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Fisheries and Environment Canada
Fisheries and Marine Service

Pêches et Environnement Canada
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Tissue Uptake, Subcellular Distribution, and Metabolism of $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$ by Rainbow Trout, *Salmo gairdneri*[1]

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OLSON, K. R., K. S. SQUIBB, AND R. J. COUSINS. 1978. Tissue uptake, subcellular distribution, and metabolism of $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$ by rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 35: 381–390.

Uptake, whole tissue, and subcellular distribution of methylmercury (MeHg) by rainbow trout, *Salmo gairdneri*, was studied during, and in three 2-wk intervals following, a single 24-h exposure to $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$. Gel permeation chromatography of cytosol fractions was performed on select tissues. Gills contained approximately 10 times as much MeHg as any other tissue after 24-h exposure whereas concentrations were lowest for brain and skeletal muscle. In the 2-wk period following exposure, gill MeHg concentrations decreased to levels comparable with most other tissues; methylmercury in most other tissues increased and then decreased during the subsequent 4-wk period. Methylmercury levels in brain, skeletal muscle, and gonad were highest at 6 wk. During the 6-wk interval following MeHg exposure, the percent of mercury as inorganic mercury (Hg^{++}) increased in gill, kidney, and liver and to a lesser extent in all other tissues except skeletal muscle. Total MeHg was greatest in cytosol fractions and usually made up from 50 to 80% of the total tissue mercury. In liver cytosol a methylmercury binding metallothionein-like species accounted for up to 40% of the total methylmercury bound. Inorganic mercury (Hg^{++}), probably derived from demethylation of methylmercury, was associated with a metallothionein-like protein detected in gill tissue but no more than 6% of the total mercury in the soluble fraction was bound to this protein(s). Mercury binding to metallothionein-like proteins in kidney and splenic fractions was minimal in spite of relatively large amounts of mercury in the cytosol from these tissues. Metallothionein may act as a binding scavenger for methylmercury and inorganic mercury in fish.

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OLSON, K. R., K. S. SQUIBB, AND R. J. COUSINS. 1978. Tissue uptake, subcellular distribution, and metabolism of $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$ by rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 35: 381–390.

Nous avons étudié l'assimilation, la distribution dans le tissu entier et la distribution subcellulaire du mercure-méthyl (MeHg) chez la truite arc-en-ciel, *Salmo gairdneri*, durant une seule exposition de 24 h à $^{14}CH_3HgCl$ et $CH_3{}^{203}HgCl$ et ensuite à trois intervalles de 2 sem consécutives. Nous avons aussi fait des analyses de chromatographie par perméation sur gel des fractions cytosol sur des tissus prélevés durant cette période. Les branchies contiennent environ 10 fois plus de MeHg que tout autre tissu après exposition de 24 h. A ce moment-là, le cerveau et les muscles squelettiques ont les teneurs les plus faibles en mercure-méthyl. Durant les 2 sem qui suivent l'exposition, les teneurs en MeHg des branchies diminuent et atteignent des niveaux comparables à ceux de la plupart des autres tissus. Le mercure-méthyl dans tous les tissus (sauf la branchie) augmente durant les 2 premières sem qui suivent l'exposition, pour diminuer ensuite dans la plupart des tissus durant la période de 4 sem subséquentes. Les teneurs en mercure-méthyl du cerveau, du muscle squelettique et de la gonade sont à leur maximum à 6 sem. Au cours de l'intervalle de 6 sem suivant l'exposition au MeHg, le pourcentage de mercure sous forme de mercure inorganique (Hg^{++}) augmente dans la branchie, le rein et le foie, et à un degré moindre dans tous les autres tissus sauf le muscle squelettique. Nous constatons que la plus faible quantité de MeHg intracellulaire est associée aux fractions mitochondriales et que la distribution entre les fractions nucléaires et microsomales est variable. Le MeHg total est à son maximum dans les fractions cytosol et se situe ordinairement entre 50 et 80% du mercure tissulaire total. Dans le cytosol hépatique, une espèce ressemblant à la métallothionéine et fixant le mercure-méthyl est responsable d'au moins 40% du mercureméthyl fixé total. Le mercure inorganique (Hg^{++}), provenant probablement de la déméthylation du mercure-méthyl, est associé à une protéine ressemblant à la métallothionéine détectée dans le tissu branchial; pas plus de 6% toutefois du mercure total contenu dans la fraction soluble est fixé à cette ou ces protéines. Il y a très peu de fixation de mercure aux protéines ressemblant à la métallothionéine dans les fractions de rein et de rate en dépit de quantités relativement grandes de mercure dans le cytosol provenant de ces tissus. Ces données suggèrent que la métallothionéine peut agir comme fixateur du mercure-méthyl et du mercure inorganique chez les poissons.

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THE anatomic and metabolic disposition of mercurials has received considerable attention in recent years primarily because of their notoriety in the environment and the consequences of toxicity in humans and animals. Inorganic and organic mercurials can be accumulated through the diet (Hannerz 1968; Jernelov and Lann 1971; Fagerström et al. 1974), by transcutaneous absorption of some mercurials from physical contact, or across respiratory surfaces (Hannerz 1968; Olson et al. 1973). Methylmercury is the predominant form of mercurial in aquatic pollution (Jernelov and Lann 1971) and, of the mercurials studied, is most readily accumulated in the tissues of fish (Hannerz 1968; Olson et al. 1973). Detoxification, following exposure to heavy metals, can proceed by direct excretion of toxic compounds via branchial, renal, or gastrointestinal routes or by detoxification in situ through conversion of the toxicant into another, less harmful form. This can be achieved by direct alteration of the primary structure of the compound through catabolism, by removing the compound from the metabolic pool by conjugation (Lech and Statham 1974), or by binding it to an existing carrier molecule with relatively high affinity for the toxicant.

Metallothionein (MT), a protein with high affinity for heavy metals, has been described in specific mammalian tissues (Kagi and Vallee 1960; Wisniewska et al. 1970; Piotrowski et al. 1973; Chen et al. 1973; Nordberg et al. 1974), avian liver (Weser et al. 1973; Bremner and Young 1976), and in the liver and kidney from the goldfish, *Carassius auratus* (Marafante et al. 1972; Marafante 1976). Although the basic function of MT is probably in the normal metabolism of zinc (Richards and Cousins 1975), the protein moiety, thionein, is also able to bind cadmium, mercury, copper, tin, and silver in addition to zinc (Sabbioni and Marafante 1975; Winge et al. 1975).

The stability of the methylmercury bond, long thought immutable, has recently been questioned. This is based in part on indirect evidence of inorganic mercury accumulation in muscle by certain species of fish (Rivers et al. 1972) and on evidence of bond splitting in mammals (Norseth and Clarkson 1970; Garcia et al. 1974a, b; Syversen 1974a, b) and in the teleosts *Lepomis macrochirus* (Burrows and Krenkel 1973) and *Fundulus heteroclitus* (Renfro et al. 1974). However, CH_3-Hg cleavage enzymes for methylmercury have not been found in rat liver or kidney (Fang 1974a;

Fang and Fallin 1974) and little methylmercury is converted to the inorganic form in the guppy (Fang 1974b).

The purpose of the present experiments was to investigate the tissue and subcellular distribution of methylmercury with respect to the lability of the methylmercury bond and to determine if a metallothionein-like molecule is involved in the detoxification process.

Materials and Methods

Fish

Rainbow trout (*Salmo gairdneri*) were obtained in the spring from the State of New Jersey, Department of Environmental Protection, Division of Fish, Game, and Shellfisheries trout hatchery in Hackettstown, New Jersey. During the experimental phase they were maintained in either fiberglass-lined wooden tanks or metal tanks (each lined with a nontoxic epoxy paint) with a continuous flow of aerated charcoal filtered well water at 12±2°C. The photoperiod was approximately 12 hL:12 hD and the fish were fed commercial trout pellets (Astra Inc., Worcester, Ma.) 3 times weekly. One week prior to dosage with the mercurials 24 fish were selected for uniform size (75–100 g) to reduce error in uptake rate (Fagerström et al. 1974) and moved to a separate tank (~1604 L). Feeding was discontinued during this period and the following 6 wk to reduce ammonia excretion (Fromm 1963) and to prevent materials in the feed from binding with mercury entrained in the enterohepatic circuit.

Fish, especially carnivores like salmonids, frequently will go for varying periods between meals. Certainly this alters enzymatic activity but it is not an unphysiological situation. Numerous investigators have shown that nonabsorbable mercury binding resins in the diet can greatly hasten removal of mercurials from an organism. If fish were fed in the present experiments, mercury loss through the enterohepatic loop could have been influenced by the amount of food each fish ate and could vary with the use of different brands of feed. Even the difference between commercial feed and that obtained in the wild could affect the results.

The day before onset of the experiment the tank was brushed to remove algal growth and water was partially drained and refilled several times to obtain the lowest possible ammonia levels and remove debris. Freshwater inflow was discontinued immediately prior to the addition of the mercurial.

Radioisotopes

Methylmercury in the form of $^{14}CH_3HgCl$ (4.17 mCi/mM) and $CH_3{}^{203}HgCl$ (500 mCi/mM) was obtained from New England Nuclear, diluted with distilled water, and mixed in the approximate ratio of 4 μCi ^{203}Hg to 1 μCi ^{14}C prior to addition to the fish tank. The final methylmercury concentration was approximately 50 nmol/L (10.7 μg/L). After 24-h exposure to the radiomercurials a group of fish was removed and processed. Freshwater inflow was resumed and within 24 h 95% of the ambient mercury was flushed from the tank into an activated charcoal–glass wool trap. This procedure obviously permitted the remaining fish to be exposed to methylmercury longer than the 24-h exposed fish and will bias the total mercury uptake values. However, the ratio of ^{203}Hg to ^{14}C should not be affected. In addition, any stress and untoward osmotic imbalances associated with moving the trout to the other tanks was avoided. The remaining fish were removed at 2, 4, and 6 wk following initial exposure to the labeled mercurials.

Tissue Preparation

Uptake rates — At the respective intervals, fish were individually removed from the tank and rinsed in a pail of fresh water for 1 min. A blood sample was collected from the hemal arch in a heparinized syringe and the fish were then killed by cervical dislocation. One-half gram (or less) of each tissue was removed and placed in a tared vial containing 3 mL of 0.4 N NaOH and reweighed to the nearest 0.5 mg. The vials were shaken until the tissues were digested, and the solution titrated back to pH 7.4 with dilute HCl (1:2, acid:water). An aliquot of the titrant was mixed with New England Nuclear Formula 947 counting cocktail and enough distilled water added to form a thick gel. The samples were counted by liquid scintillation spectrometry.

Subcellular fractionation — Tissue samples, 1 g or less, were added to 9 vol of cold (4°C) 0.25 M sucrose buffer–Tris–HCl (TKM, 0.05 M Tris–HCl, 5 mM $MgCl_2$, 0.025 M KCl; pH 7.4, Cousins et al. 1970). The tissue was homogenized while on ice with a motor driven, Potter-Elvehjem type glass–teflon homogenizer. The crude nuclear fraction was isolated by centrifugation in a Sorvall refrigerated centrifuge for 10 min at 1000 × *g*. The pellet was resuspended in buffer and recentrifuged as above. The pellet was dissolved in 0.4 N NaOH and prepared for liquid scintillation counting as described. The supernatant and nuclear wash were combined and the mitochondrial fraction isolated by centrifugation at 10 000 × *g* for 10 min. The mitochondrial fraction was resuspended once and then dissolved in NaOH and prepared for counting as above. The postmitochondrial supernatants were combined and the microsomal and soluble cytosol fractions separated by ultracentrifugation at 166 000 × *g* for 1 h in a Beckman ultracentrifuge (Feldman and Cousins 1976). The microsomal pellet was dissolved in NaOH and counted for radioactivity as above. An aliquot of the postmicrosomal supernatants was added directly to the counting cocktail. Aliquots of all subcellular fractionations were analyzed for protein by the method of Lowry et al. (1951).

An aliquot of the cytosol was fractionated on a 2.5 × 50 cm column of Sephadex G-75 equilibrated and eluted with 10 mM Tris–HCl (pH 8.6). This procedure yields an MT fraction that is at least 85% pure (Feldman and Cousins 1976). The absorbance at 280 nm of each fraction (5 mL) was measured and 3 mL of each fraction was added to a counting cocktail and counted, as a gel, by liquid scintillation spectrometry.

Separation of $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$ — Since the spectra of ^{14}C and ^{203}Hg are fairly similar (Cousins et al. 1975) and considerable quenching was expected from blood and kidney homogenates, the two isotopes were separated on the basis of half lives rather than by differential liquid scintillation counting. The rationale and efficacy of this method is described in detail elsewhere (Olson 1977). Standards containing single isotopes were prepared with various activities and added to serially diluted tissue digests from unlabeled (control) fish. Quench correction curves for each isotope were derived from these samples by the ex-

TABLE 1. Methylmercury uptake (nanomoles of total methylmercury per gram tissue wet weight ± SE) by tissues of rainbow trout (*Salmo gairdneri*) after exposure to $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$. Number in parentheses is number of fish in each group.

Tissue	Time after initial exposure			
	24 h (5)	2 wk (5)	4 wk (5)	6 wk (4)
Blood	9.6±0.9	60.5±18.1	32.0±1.5	38.6±1.6
Heart	7.1±0.8	11.6±1.0	7.3±0.9	8.8±0.3
Spleen	14.1±2.0	71.6±7.1	28.4±0.7	24.9±2.4
Gonad	1.9±0.4	6.1±1.4	2.7±0.3	18.1±0.4
Liver	8.5±1.5	24.0±2.3	18.3±0.9	17.1±1.6
Gill	100.1±10.8	14.6±0.8	6.7±0.2	7.5±0.1
Stomach	3.8±0.5	13.8±3.6	7.6±0.5	8.2±0.1
Cecum	6.6±0.8	15.4±1.4	12.5±0.7	11.7±0.8
Intestine	6.4±0.8	14.9±1.0	12.1±1.3	10.6±0.7
Brain	1.2±0.1	5.7±0.4	4.2±6.4	7.0±0.9
Kidney	16.1±1.6	20.9±1.4	13.5±1.2	12.8±1.7
Skeletal muscle	0.8±0.1	5.4±0.1	5.0±0.2	7.5±0.8

TABLE 2. Tissue binding of methylmercury corrected for blood borne mercurials within tissue vascular space. Number in parentheses is percent of total tissue activity due to blood bound mercurials within the vascular space.

Tissue	Blood space[a] (g/100 g tissue)	Time after initial exposure			
		24 h	2 wk	4 wk	6 wk
Spleen	3.6	13.7[b](2)	69.4 (3)	27.2 (4)	23.5 (6)
Liver	8.9	7.6 (10)	18.6 (22)	15.5 (16)	13.7 (20)
Gill	17.0	98.4 (2)	4.3 (70)	1.3 (81)	0.9 (87)
Kidney	16.5	14.5 (10)	10.9 (48)	8.2 (39)	6.4 (50)
Muscle	0.4	0.8 (5)	5.2 (5)	4.9 (3)	7.4 (2)

[a]From Hoffert 1966.
[b]Tissue bound activity = total activity (tissue and blood) − calculated blood activity.

ternal standard channels ratio (ESCR) method using a Beckman LS 150 liquid scintillation spectrometer.

Between counting periods the samples were stored at 4°C and prior to each counting were warmed at 40°C for 2 h and shaken to resuspend any settled particulate matter. Because of extreme color quenching in blood, spleen, and kidney digests, these tissues had to be diluted several times to obtain samples with satisfactory ESCR.

The Student's *t*-test was used for all statistical comparisons.

Results

Total Methylmercury Uptake

The uptake of methylmercury in tissues (Table 1) follows a typical pattern previously described in fish (Hannerz 1968; Jernelov and Lann 1971; Olson et al. 1973; Renfro et al. 1974). In the 2- and 6-wk period, however, many tissues demonstrated changes in total mercury levels that were qualitatively if not quantitatively similar to blood values, suggesting that part of this phenomenon was due to mercury in blood trapped within the tissues. Corrections for mercury bound to blood were made for several tissues using blood volume values from Hoffert (1966) determined on another salmonid, the lake trout, *Salvelinus namaycush* (Table 2). Tissue mercury levels are highly variable: the greatest rate of mercury loss is from the gill and the lowest rate of loss from the liver. However, skeletal muscle levels continue to increase throughout the experimental period.

Stability of CH_3-Hg Bond in Tissues

The stability of the CH_3-Hg bond was determined by the ratio of ^{203}Hg dpm to ^{14}C dpm in the tissue digests (Table 3). This procedure suffers from the limitation that said ratio will not change if the mercury is demethylated and both products remain within the tissue. The likelihood that tissue turnover rates will be identical for both isotopes seems small; thus, even though the stoichiometry of the bond cleavage can not be ascertained, insights into tissue specificities for bond cleavage or product turnover can be realized.

Ratios from 24-h exposure were relatively uniform

TABLE 3. Ratio of ^{203}Hg to ^{14}C activity in tissues of rainbow trout following exposure to a single pulse of ^{203}Hg- and ^{14}C-labeled methylmercury. Number in parentheses is number of fish in each group.

	Mean $\frac{\text{dpm } ^{203}Hg}{\text{dpm } ^{14}C} \pm$ SE			
	Time after initial exposure			
Tissue	24 h (5)	2 wk (5)	4 wk (5)	6 wk (4)
Blood	2.82±0.233	3.04±0.215	3.77±0.051	3.69±0.059
Heart	3.68±0.094	4.16±0.106	4.30±0.115	4.65±0.059
Spleen	3.11±0.175	2.82±0.273	4.09±0.316	4.46±0.370
Gonad	3.47±0.107	4.05±0.122	4.47±0.260	4.73±0.416
Liver	3.56±0.062	4.46±0.069	4.43±0.135	5.30±0.567
Gill	3.42±0.094	6.21±0.137	7.57±0.182	8.35±0.224
Stomach	3.76±0.038	4.14±0.121	4.24±0.087	4.48±0.043
Cecum	3.68±0.112	4.16±0.093	4.23±0.037	4.70±0.110
Intestine	3.65±0.052	4.29±0.044	4.32±0.057	4.61±0.275
Brain	3.67±0.151	4.63±0.125	4.75±0.177	4.54±0.198
Kidney	3.60±0.095	4.35±0.050	5.65±0.430	6.50±0.386
Skeletal muscle	3.74±0.128	3.99±0.102	3.86±0.041	3.97±0.233

with the exception of significantly ($P < 0.05$) lower values for blood and spleen, the latter probably reflecting its blood and/or trapped red cell volume. Gill tissue with a considerable blood volume also had a smaller ratio, while kidney with a blood volume similar to that of the gill demonstrated a ratio similar to other tissues, an indication of specific renal uptake of methylmercury. Ratios increased for all tissues except skeletal muscle during the 6-wk period following mercury exposure.

METHYLMERCURY IN BLOOD

The percent ^{203}Hg and ^{14}C associated with the cellular fraction of heparinized whole blood as well as the ratios of these radionuclides in both the cellular and plasma fractions were determined on five blood samples from fish collected at the 6-wk period. The percent ^{203}Hg incorporated into red cells (± SE) was 55.9 ± 4.90, a value not statistically different from that for ^{14}C incorporation, which was 61.2 ± 3.38. Ratios of ^{203}Hg to ^{14}C were 3.55 ± 0.366 for red cells and 4.51 ± 0.362 for plasma ($P \leq 0.10$).

INTRACELLULAR DISTRIBUTION OF MERCURY

Intracellular distribution of ^{203}Hg and ^{14}C for single or pooled tissues at their respective intervals is shown in Fig. 1. As marker enzymes were not identified for each fraction, the separation of organelles is only approximate. Several trends are evident, however, and in general the average tissue ratios of ^{203}Hg:^{14}C from the fractions correspond quite well to total tissue ratios from Table 3. Since mercury of one form or another has been shown to bind or affect many subcellular components including nuclei (Bryan et al. 1974), mitochondria (Syversen 1974a; Southard et al. 1974), lysosomes (Norseth and Brendeford 1971; Fowler et al. 1974; Fowler et al. 1975), and the cytosol fraction either bound to protein or unbound (Norseth and Brendeford 1971; Bush et al. 1973; Nordberg et al. 1974), it was not surprising to find mercury associated with many of these fractions in the present experiments.

Ratios of ^{203}Hg:^{14}C for subcellular fractions were similar to ratios calculated for whole tissue (Table 3) and increased toward the end of the experiment.

The total mercury ($^{14}CH_3Hg + CH_3{}^{203}Hg$) concentrations in the cytosol of various tissues are shown in Table 4. No corrections were made for inorganic mercury in the tissues.

Methylmercury incorporation into the cytosol of liver, ceca, and kidney increased steadily for at least 4 wk at which time these values were approximately 50 times greater than the methylmercury content of skeletal muscle cytosol. However, the methylmercury content of these tissues on a wet weight basis differed by only a factor of 4.

CYTOSOL CHROMATOGRAPHY

Chromatography of the cytosol fractions was done at various times after the methylmercury pulse to explore the chronology and general types of ligands responsible for methylmercury binding (Tables 4 and 5). Most of the ^{203}Hg and ^{14}C activity was associated with fractions of high molecular weight (>75 000 daltons; approximately fraction no. 17–27) in all tissues. No appreciable radioactivity was found in the lower molecular weight (mw) fractions (between 6000 and 10 000 daltons and which contained MT) in gill or kidney cytosol after 24 h of methylmercury exposure. Only minor MT peaks, barely above background, were observed in these same fractions for spleen and kidney

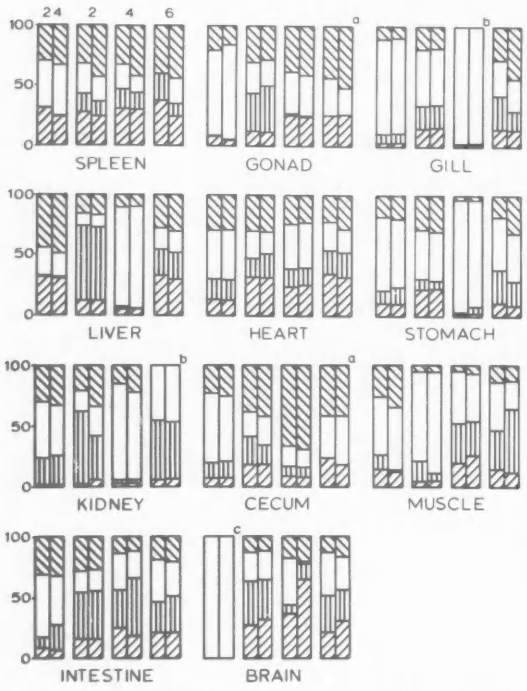

FIG. 1. The percent of ^{203}Hg and ^{14}C in subcellular fractions of rainbow trout 24-h, 2, 4, and 6 wk after exposure to a pulse of $CH_3{}^{203}HgCl$ and $^{14}CH_3HgCl$. The left column of each pair represents ^{203}Hg radioactivity and the right one ^{14}C. Data were obtained by dividing the radioactivity of either ^{203}Hg or ^{14}C for each subcellular fraction by the total activity of the specific radioisotope in the tissue. Lines slanted to right (bottom of column) = nuclear fraction; vertical lines (second from bottom) = mitochondrial fraction; unmarked = microsomal fraction; slanted to left (top of column) = cytosol fraction. Single or pooled tissue samples. a, Mitochondrial fraction not available; b, cytosol fraction not available; c, 24-h data not available.

TABLE 4. Total methylmercury (nanomoles methylmercury per gram protein) in the cytosol fraction of various tissues as a function of time. Values are for single fish or pooled samples. ND = not determined.

	Time			
Tissue	24 h	2 wk	4 wk	6 wk
Spleen	0.80	2.91	2.77	2.35
Ovary	0.24	0.82	0.61	0.44
Gill	13.40	1.14	ND	1.19
Liver	0.67	1.76	5.24	1.10
Heart	0.79	1.01	0.62	0.62
Stomach	0.29	0.82	0.75	0.72
Kidney	1.53	1.74	5.61	ND
Cecum	1.10	2.59	5.19	1.14
Skeletal muscle	0.07	0.24	0.11	0.42
Intestine	1.15	1.73	0.46	0.69
Brain	ND	0.25	0.21	0.50

TABLE 5. Ratio of ^{203}Hg to ^{14}C in chromatographed post-microsomal supernatant fractions from trout exposed to $CH_3{}^{203}HgCl$ and $^{14}CH_3HgCl$. Ratio calculated from fractions with maximal activity. (—) indicates no samples above 5 times background.

Tissue exposure	Large molecular weight	Metallothionein
Spleen (2 wk)	5.02, 3.67	—
(6 wk)	3.05	—
Gills (24 h)	3.80, 3.67	—
(2 wk)	9.22, 4.33	11.9
(6 wk)	5.37, 2.82	9.12
Liver (24 h)	3.05, 2.63	2.06
(2 wk)	4.91	2.40
(4 wk)	3.63	6.23
(6 wk)	4.22, 4.10	3.99
Kidney (24 h)	3.38, 3.45	—
(2 wk)	10.08, 6.50	—
(6 wk)	4.76, 3.75	—

cytosol 2 and 6 wk after exposure. The small MT peak found in the kidney after 6 wk was accounted for primarily by ^{203}Hg. The radioactivity in MT fractions never exceeds 5% of the radioactivity associated with the high mw fractions and usually was under 2%.

Chromatography of gill cytosol taken 2 and 6 wk (Fig. 2A) after exposure to methylmercury revealed a prominent ^{203}Hg-containing MT peak with very little radioactivity due to ^{14}C. This fraction contained 6% of the soluble ^{203}Hg in gills 2 wk after exposure and 20% after 6 wk of exposure.

Chromatography of the cytosol of livers from trout collected at all time periods demonstrated significant incorporation of both ^{14}C and ^{203}Hg into MT. The total radioactivity in this fraction (^{14}C plus ^{203}Hg) compared to the high molecular weight fractions was 20, 41, 8, and 7% for tissues taken at 24 h, 2 wk, 4 wk, and 6 wk, respectively. Figure 2B shows the distribution of ^{14}C and ^{203}Hg in cytosol from 4-wk liver which was qualitatively similar to all liver samples.

The ratio of ^{203}Hg to ^{14}C in chromatographic fractions from the various tissues is shown in Table 5. In general, the high mw fraction contained methylmercury (^{14}C and ^{203}Hg) whereas the MT fraction was mostly inorganic mercury (^{203}Hg) in gill and kidney and methylmercury (^{14}C and ^{203}Hg) in liver.

Discussion

Tissue mercury concentration following methylmercury exposure demonstrates selective tissue binding and subsequent redistribution of both methyl- and demethylated mercury (Tables 1–3). All tissues except

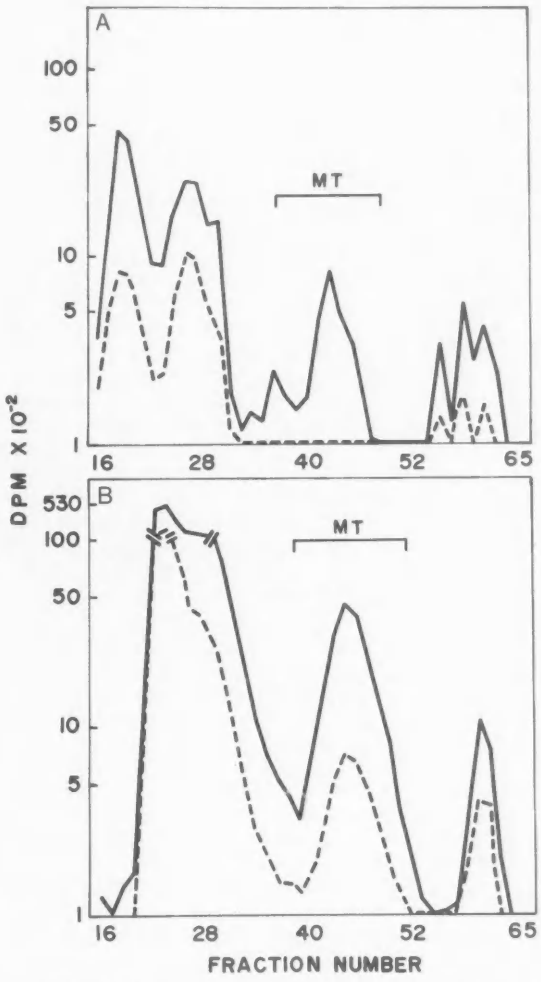

FIG. 2. A, Chromatography of trout gill cytosol. Tissues were taken 6 wk after a 24-h pulse of $^{11}CH_3HgCl$ and $CH_3{}^{203}HgCl$. MT, approximate metallothionein peak; solid line, ^{203}Hg activity; dashed line, ^{11}C activity. B, Chromatography of trout liver cytosol. Tissues taken 4 wk after a 24-h pulse of $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$. Notation as in Fig. 2A.

brain, skeletal muscle, and gonad lost mercury in the 2- to 6-wk interval after exposure. The calculated ratio of liver:muscle mercury concentrations confirms that projected by Jernelov and Lann (1971) and reflects the status of mercury uptake; i.e. as mercury is accumulated by the fish an increase in liver concentrations will precede skeletal muscle levels and, when mercury is no longer taken up from the environment, the fall in liver mercury will again precede the fall in skeletal muscle mercury content.

During initial 24-h exposure, gill filaments exhibit the highest mercurial concentrations exceeding the kidney by a factor of 6.2. Gill mercury concentrations fall dramatically 2 wk after removal of the methylmercury to levels below most other tissues. This is indicative of two processes, sloughing of mercury bound to gill mucins (McKone et al. 1971; Olson and Fromm 1973) and rapid permeation of mercury through gill epithelia. The latter is supported by the transient nature of sodium transport inhibition by mercury in the killifish (Renfro et al. 1974).

The very low "blood free" mercury levels in gills at weeks 2, 4, and 6 (Table 2) is quite striking in that the apparent tissue concentration in the gills is nearly an order of magnitude lower than any other tissue. A discrepancy can be noted here between gill total methylmercury levels corrected for blood mercury (Table 2) and ^{203}Hg:^{14}C ratios (Table 3). In the former calculations, after 6 wk, 87% of the total mercury is due to blood. If this were true, the maximum ratio obtainable, assuming the remaining 13% bound to gill tissue was inorganic mercury, would be 3.84 which is far lower than the 8.35 observed (Table 3). The best explanation for this descrepancy is an overestimation of gill blood volume which is entirely possible if the fish are stressed such as by handling or by starvation. Calculations similar to the above show that maximum ratios for kidney, liver, muscle, and spleen (assuming all tissue bound mercury is inorganic) are all larger than the experimental ratios, thus the tissue corrected data seems to have greater validity in these tissues. Although extrapolating data on blood volumes from one species of fish to another is tenuous, it does point to the necessity of this type of correction whenever tissue distribution of any compound is studied.

In general, the total mercury levels in subcellular fractions (data not presented) parallel whole tissue mercury concentrations and the pattern of intracellular mercury distribution seemed to be tissue specific (Fig. 1). There did not appear to be any chronological trends in the percent mercury bound to subcellular fractions. The percent mercury in samples uncorrected for protein was greatest in cytosol and varied between 50 and 80% of the total (data not presented); however, when they were normalized to a constant protein content the percent mercury in cytosol decreased to between 20 and 45% for most samples. The failure to observe any consistent ratio changes between the cellular fractions for any tissue is indicative of an intracellular equilibrium with each specific fraction gaining or losing organic (and inorganic) mercury in concert with total cellular levels. Thus as the ratio increases for gill tissue in general, so it increases for each subcellular fraction. Changes in the ratios of ^{203}Hg to ^{14}C activity in various tissues (Table 3) not only demonstrate redistribution of these molecules but reflect the metabolic disposition of demethylation products and suggest sites of demethylation and perhaps excretion. Of the tissues examined, gill, liver, kidney, gastrointestinal tract, and

spleen are the most probable sites for demethylation and/or excretion.

During the last 4-wk period, heart, gonad, stomach, ceca, and intestine lost total mercury while the ^{203}Hg:^{14}C ratio only slightly increased (Table 3). This implicates methylmercury as the major species lost with some inorganic mercury remaining in these tissues. Ratios for brain (Table 3) increased from 24 h to 2 wk suggesting increases in inorganic mercury as in several other tissues. However, stabilization of ratios as observed during the remaining 4 wk, in spite of increasing total mercury levels, indicates slow increases of methylmercury. This is even more evident for skeletal muscle where tissue ratios remained constant while tissue levels steadily increased.

The most significant increases in ratios during this period are in the gill, kidney, and to a lesser extent the liver. The latter two have been implicated in the demethylation process in teleosts (Burrows and Krenkel 1973) and other vertebrates (Norseth and Clarkson 1970) and the present data support this contention. Changes in kidney and to a lesser extent liver ratios relative to total mercury concentrations can therefore be explained by loss of methylmercury and carbon moities from demethylation and retention of mercury in the inorganic form.

The function of the gill in demethylation and/or excretion of methylmercury can only be speculated upon; high ratios adjunct with very low total tissue levels suggest excretory capabilities for methylmercury. Since gills appear to actively secrete other assorted organic molecules such as *p*-aminohippurate (PAH) (Masoni and Payan 1974), gill excretory capabilities for methylmercury are certainly feasible. Gill tissue lost mercury (total) after 24 h in spite of increased ratios (almost twofold). Thus the gill appears to be losing ^{14}C from demethylation, although it is conceivable that the gill is losing ^{14}C and ^{203}Hg as methylmercury and retaining ^{203}Hg from an inorganic mercury source such as microbial demethylation in the water.

Loss of mercury from the spleen is chronologically similar to mercury loss from blood (Tables 1 and 3). Since much of the mercury is initially bound to the red cells (Olson and Fromm 1973), this decrease could reflect turnover or catabolism of red cells sequestered within the spleen. The fate of mercury during red cell phagocytosis by the spleen is unknown but this could be a contributory mechanism in mercury detoxification and redistribution. During the 4 wk after exposure, the ratios of ^{203}Hg:^{14}C in spleen and blood (Table 3) remained lower than other tissues; however, after 6 wk the spleen ratio approximated that for many other tissues and was substantially greater than the blood ratio. This also suggests loss of mercury concomitant with loss of red cells from the spleen. The large mercurial component found in the plasma has not been observed following methylmercury exposure in short-term in vitro experiments in trout blood (Olson and Fromm 1973; Giblin and Massaro 1975) or rabbit blood (Garrett and Garrett 1974). In all previous studies about 90% of the methylmercury has been found in the cellular fraction. The difference in cell plasma ratios indicates mercury in the cellular fraction remains methylated while the plasma contains relatively more mercury in the inorganic form.

Renfro et al. (1974) found evidence for demethylation from ^{14}C:^{203}Hg whole body ratios in groups of *Fundulus* exposed for 24 h to either isotope in the methylmercury form. These authors reported a decrease in the ^{14}C:^{203}Hg ratio from 1.72 to 1.07 in the 5- to 117-h period following removal of the mercurials. This corresponds to an increase in the ^{203}Hg:^{14}C ratio from 0.58 to 0.93 in 4.87 days, a much greater change than observed in the present experiments with rainbow trout. Because Renfro et al. (1974) measured whole body activity, either skeletal muscle was involved in the demethylation process or, if not, tissues such as gills, kidney, and liver would have to lose ^{14}C at even greater rates. On the other hand, the percent of total mercury as methylmercury in whole bluegills, *L. macrochirus*, has been reported to remain constant up to 95 days after mercurial exposure (Burrows and Krenkel 1973). The authors also reported a substantial decrease in the percent of methylmercury in liver and kidney within 10 days and by 30 days only 10% of the total mercury in these organs was methylmercury compared to a whole body percentage of 73. This suggests an increase in the percent methylmercury in another organ to offset liver and kidney decreases. Data reported in the present experiment showing continual methylmercury uptake by brain and skeletal muscle tissues support this hypothesis. The differences in rates of demethylation and tissue binding between *F. heteroclitus*, *L. macrochirus*, and *Salmo gairdneri* shows substantial species specificity in the methylmercury detoxification process. Complete understanding of this process in teleosts will obviously require investigation of a variety of species with varying environmental and physiological identities.

Metallothioein (MT) bound mercury has been reported in kidney cytosol from other vertebrates in significantly greater amounts than reported in the present study. Nordberg et al. (1974) described an MT-like protein in renal tissue from rabbits exposed to high levels of inorganic mercury (Hg^{++}) that had high binding affinity for mercury and contained substantial amounts (75%) of the total soluble mercury. Marafante (1976) reported that approximately 30% of the soluble mercury was bound to MT in kidneys from goldfish injected with $HgCl_2$. The discrepancy between these studies and the present one could be due to species specificity or, more probably, different detoxification mechanisms for methylmercury and inorganic mercury. Obviously the capability for MT induction following inorganic as opposed to methylmercury exposure, as well as specific concentrations required, could differ greatly thus accounting for the minimal Hg–MT binding observed in the present study.

Even though mercury is probably demethylated in the trout kidney it is doubtful if this could account for enough inorganic mercury to induce metallothionein synthesis. In rats there is a continuous low level synthesis of thionein (Squibb and Cousins 1974) and this rate of synthesis can be enhanced by cadmium or zinc. We believe the renal Hg–MT detected in trout, which were exposed to minimal amounts of mercury, is a reflection of an accumulation of thionein, originally involved in zinc homeostasis and synthesized at a low rate. Upon demethylation, the Hg^{++} replaces the native metal on this ligand, zinc. Methylmercury is not bound by MT in renal tissue of the trout, as evidenced by lack of ^{14}C activity in the MT fraction.

Gill MT could represent a mechanism for removal or detoxification of mercury (probably in the inorganic form) as up to 20% of the soluble mercury appeared bound to this fraction. It is noteworthy that the ratio of ^{203}Hg to ^{14}C in gill MT was about 10 while the ratio was about 4 for nearly all the high mw fractions (Table 5). This indicates that mercury was demethylated before being bound to MT. The MT-like species in gill and renal cytosol migrate identically on a calibrated column and thus are probably the same moiety. (In this light, it would be interesting to assess the role of the gill in zinc metabolism in teleosts.)

The greatest amount of Hg–MT was found in liver cytosol, and this tissue probably has the greatest potential for autodetoxification of mercury by metallothionein synthesis. The ratio of ^{203}Hg to ^{14}C in MT from liver cytosol was constant and consistent with the ratio in whole liver and indicates that methylmercury is bound to MT intact. Appearance of both ^{14}C and ^{203}Hg in MT appeared to reach a maximum between 24 h and 2 wk. An alternative that could explain the presence of ^{14}C in MT could be that ^{14}C from demethylation was incorporated into liver proteins via the synthesis of nonessential amino acids. This, however, would require near stoichiometric incorporation of ^{14}C methyl groups, as amino acids, and ^{203}Hg as Hg^{++}, into the protein. Since the liver does not appear to readily demethylate methylmercury in quantities commensurate with this alternative, bond cleavage and reincorporation in this organ seems unlikely. It is not known why gill and kidney MT bind inorganic mercury whereas methylmercury is predominately associated with liver MT. Even through metallothioneins are present in fish, in the tissues examined, MT does not account for the majority of mercury bound in the soluble fractions. This observation raises the question that perhaps Hg^{++} replaces Zn^{++} in thionein, since the basic function of MT is probably related to the latter cation (Feldman and Cousins 1976) and mercury is more tightly bound to metallothionein (Pulido et al. 1966). If induction of metallothionein synthesis is insufficient to ameliorate the mercury burden, displacement of native zinc by mercury could have significant consequences on zinc homeostasis.

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Comparative Recoveries of Planted Yearling and Fall-Fingerling Brook Trout (*Salvelinus fontinalis*) from Ontario Lakes[1]

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FRASER, J. M. 1978. Comparative recoveries of planted yearling and fall-fingerling brook trout (*Salvelinus fontinalis*) from Ontario lakes. J. Fish. Res. Board Can. 35: 391–396.

Yearling and fall-fingerling brook trout (*Salvelinus fontinalis*) were planted in two lakes with few or no resident fish species and in three lakes with resident populations. The comparative recoveries of the two size-groups of trout planted were assessed by creel census and/or intensive gillnetting. Recoveries of planted yearling brook trout were greater from lakes supporting few or no resident fish species (15.9–35.9%) and less in lakes supporting suckers, minnows, and sticklebacks (5.9–14.8%). Experience was similar with fall fingerlings planted in the same set of lakes though comparative recoveries were less. Lakes with few fish competitors yielded 3.5–4.8 kg of trout for each kilogram of fall fingerlings planted compared with 2.9–3.0 kg for each kilogram of yearlings planted. The returns of trout from lakes also supporting resident species were only 0.3–0.8 kg for each kilogram of yearlings or fall fingerlings planted.

Key words: Salvelinus fontinalis, planting, survival, growth, yield

FRASER, J. M. 1978. Comparative recoveries of planted yearling and fall-fingerling brook trout (*Salvelinus fontinalis*) from Ontario lakes. J. Fish. Res. Board Can. 35: 391–396.

Des truitelles de l'année et des fingerlings d'automne de l'omble de fontaine (*Salvelinus fontinalis*) furent introduits dans deux lacs contenant peu ou pas d'espèces de poissons résidents et dans trois lacs possédant des populations résidentes. Nous avons comparé les retours des deux groupes d'ombles ensemencés au moyen d'un recensement au panier et/ou d'un échantillonnage intensif au filet maillant. Les retours d'ombles de fontaine de l'année introduits sont plus nombreux dans les lacs contenant peu ou pas d'espèces de poissons résidents (15.9–35.9%) et moins nombreux dans les lacs contenant des castostomidés, des ménés et des épinoches (5.9–14.8%). Il en est de même des fingerlings d'automne introduits dans les mêmes groupes de lacs, bien que les retours comparatifs aient été moindres. Les lacs contenant peu de compétiteurs donnent 3.5 à 4.8 kg d'ombles de fontaine par kilogramme de fingerlings d'automne ensemencé, comparativement à 2.9–3.0 kg par kilogramme de truitelles de l'année ensemencées. Les retours d'ombles de fontaine dans les lacs contenant également des espèces résidentes ne sont que de 0.3–0.8 kg par kilogramme de truitelles de l'année ou de fingerlings d'automne ensemencés.

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SOME 1.2 million hatchery-reared brook trout (*Salvelinus fontinalis*) are planted annually in several thousand lakes in Ontario. Approximately 85% of these fish are planted as yearlings (16 mo) and the remainder as fall fingerlings (9 mo). Since the cost of rearing yearling brook trout in Ontario hatcheries is approximately 3 times the cost of fall fingerlings (D. Sinclair, Ontario Ministry of Natural Resources, personal communication) it is essential to know the relative rates at which the two size-groups are recovered subsequent to planting.

There has been considerable study directed at comparing the results of spring and fall planting in streams (Cooper 1959; Needham 1959) but similar information on lake plantings has pertained mainly to reclaimed waters (Wales and German 1956; Hatch and Webster 1960) or otherwise managed waters (Smith 1952a, 1956).

This study compares the recoveries of planted yearling and fall fingerling brook trout from lakes categorized according to fish fauna: Myra, barren; Little Mykiss, minnows; Crevice, Major, and Sawlog, white suckers (*Catostomus commersoni*), brook stickleback (*Culaea inconstans*), and 3–5 minnow species. Myra and Little Mykiss lakes are small dystrophic lakes which have not been angled because of difficult access to their locations in Algonquin Park. Crevice, Major, and Sawlog lakes, located just outside the southeast boundary of the park, were fished throughout the angling season, which extended from January 1 to September 30. The basic productivities of the lakes are thought to be roughly similar, as suggested by the physical and chemical characteristics (Table 1).

[1]Ontario Ministry of Natural Resources, Fish and Wildlife Research Branch, Contribution No. 77-12.

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TABLE 1. Characteristics of study lakes (number of determinations in parentheses).

Lake	Area (ha)	Max depth (m)	Mean depth (m)	Secchi disc visibility (m)	pH	Total alkalinity (mg/L)	Total dissolved solids (mg/L)
Myra	5.5	8.5	3.0	4.5 (10)	6.3 (8)	8 (5)	28 (5)
Little Mykiss	9.3	6.7	2.7	2.1 (7)	6.5 (4)	9 (3)	47 (3)
Crevice	25.5	18.3	6.1	4.9 (31)	6.8 (8)	11 (6)	44 (6)
Major	26.3	14.6	7.3	4.8 (12)	7.1 (5)	15 (4)	54 (4)
Sawlog	6.3	8.5	4.3	4.7 (11)	6.9 (7)	9 (4)	31 (4)

Methods of Planting and Recovering

Fall fingerlings were planted annually at rates ranging from 32 to 134 fish/ha in Myra and Little Mykiss lakes (Table 2). In Crevice and Major lakes the rate was relatively constant at 62–78 fish/ha and in Sawlog Lake at 254 fish/ha. Yearlings were planted at comparable rates but did not exceed 130 fish/ha in any lake. Additionally, Crevice and Major lakes received plantings of F_1 splake (*S. namaycush* × *S. fontinalis*) as part of a companion study (Fraser 1972).

The trout were reared at Pembroke Provincial Hatchery from eyed eggs produced by domestic stock at Hills Lake Hatchery. Prior to distribution the fish were finclipped, counted into lots, and retained for 1–5 d. MS-222® (ethyl *m*-aminobenzoate methanesulfonate) was used to anesthetize the fish prior to finclipping. Samples of fish were dipnetted from rearing troughs and fork lengths were measured to the nearest 2.5 mm (Table 2). Mean weights were determined by counting the number of fish in 2.3-kg lots.

The fish planted in Crevice, Major, and Sawlog lakes were transported by truck in cool, oxygenated tanks. Myra and Little Mykiss lakes were planted by airdrop. Fish-carrying equipment and loading rates were as described by Fraser (1968). Yearlings (19–46 g) were planted from late April to late May, and fall fingerlings (8–11 g) from late September to late October. The study extended over a period of 8 yr in Crevice Lake and 4 yr in Sawlog Lake.

Crevice, Major, and Sawlog lakes were visited twice daily by a creel census worker during the open-water season and in winter on one weekend day and three weekdays per week, chosen at random (Fraser 1972). On this basis, I estimate that the creel census covered about 75% of the angling in these lakes and accordingly angling effort and catch have been multiplied by a factor of four thirds to provide estimates of total angling returns. Although anglers accounted for a large percentage of the fish recovered, a heavy exploitation was ensured by gillnetting in August after the angling fishery had subsided (Table 3).

Myra and Little Mykiss lakes were not angled during the study and trout were recovered by intensive gillnetting with standard net gangs each August and September (Table 3). The standard gillnet gang consisted of four monofilament panels (38-, 51-, 64-, and 76-mm mesh, stretched measure) each measuring 1.8 m deep and 15.2 m long.

All fish caught in the gillnets were brought to the laboratory for examination. Fish caught by anglers were measured (fork length) and examined for finclips in the field. Weights of angled fish were estimated from length–weight curves for the respective lakes.

Results

Recoveries of Planted Trout

Yearlings were recovered in greater proportion than fall fingerlings in all five lakes (Fig. 1). The highest mean recovery was 35.9% from six annual plantings of yearlings in Myra Lake, which was devoid of other fish (Table 2). The next highest recovery was 15.9% from Little Mykiss Lake, which supported a minnow population. In the three lakes supporting suckers, minnows, and sticklebacks the mean yearling recoveries were 14.8% in Crevice Lake, 11.5% in Sawlog Lake, and 5.9% in Major Lake.

Fall-fingerling recoveries followed a similar pattern, although the differences in recoveries among the lakes were much smaller (Fig. 1). Myra and Little Mykiss lakes returned 10.5 and 10.7%, respectively, of fall-planted trout. The mean fingerling recoveries in the three sucker-supporting lakes ranged from 6.9 to 4.2%.

There was greater variability in the recoveries of yearlings compared with fall fingerlings in all lakes (Fig. 1). There was also greater variability in the recoveries of yearlings and fall plantings from the monoculture of trout in Myra Lake than was noted in the more complex fish communities inhabiting Crevice, Major, and Sawlog lakes.

Trout were planted at several rates in Myra and Little Mykiss lakes but no relationship was found between the number planted and the percentage numerical return for either yearlings or fall fingerlings. A relationship does exist, however, between planting rate and weight caught for yearlings, as will be described later.

Weight Caught to Weight Planted

The ratios of weight of trout caught to weight planted indicate a significant difference between the lakes supporting suckers and those lacking suckers. Each kilogram of trout planted in lakes supporting suckers yielded on the average only 0.3–0.8 kg of trout in return, while lakes lacking suckers returned 2.9–4.8 kg of trout (Fig. 1).

The weight-recovery ratio is dependent on the survival rate, growth rate, and time interval between release and recovery of the fish. In Fig. 2 the growth of fingerlings planted in the fall of 1963 is compared with

TABLE 2. Recoveries by angling and gillnetting of planted brook trout from the study lakes (in the order fall fingerlings and yearlings). Values in parentheses below the year of planting are mean fork lengths (cm) at planting.

	Year of planting							
	1962 (8.9) (11.9)	1963 (9.4) (11.9)	1964 (9.9) (11.9)	1965 (9.7) (14.0)	1966 (9.4) (14.2)	1967 (9.9) (13.7)	1968 (9.1) (15.7)	1969 (15.5)
				Myra Lake				
No. planted/ha	55	109	109	55	109	109	—	—
	—	55	27	55	55	109	109	
Wt planted (kg)	1.8	5.0	6.4	3.2	5.4	7.7	—	—
	—	4.5	2.7	9.1	9.5	15.9	27.2	—
Wt. caught (kg)	11.2	64.5	1.3	13.4	24.3	7.7	—	—
	—	18.0	14.3	30.0	15.2	27.0	57.1	—
				Little Mykiss Lake				
No. planted/ha	134	43	130	43	130	—	32	—
	65	22	65	22	65	—	130	—
Wt planted (kg)	7.7	3.2	12.7	4.5	10.9	—	2.3	—
	9.1	3.2	10.9	5.9	19.5	—	54.4	—
Wt caught (kg)	17.7	19.5	30.5	19.8	33.8	—	6.7	—
	40.0	16.0	40.3	7.7	37.1	—	54.4	—
				Crevice Lake				
No. planted/ha	62	62	62	62	62	78	78	—
	62	62	62	94	62	79	59	59
Wt planted (kg)	10.4	13.2	16.8	18.1	14.5	25.9	22.2	—
	24.1	24.1	29.1	72.6	51.8	53.1	68.1	84.9
Wt caught (kg)	16.6	11.9	11.8	5.4	5.8	15.5	6.7	—
	19.3	16.9	23.3	43.6	10.4	31.9	34.1	34.0
				Major Lake				
No. planted/ha	62	62	62	62	—	—	62	—
	62	62	62	62	—	—	62	—
Wt planted (kg)	11.3	11.3	16.8	16.8	—	—	17.7	—
	24.1	24.1	29.1	48.6	—	—	72.6	—
Wt caught (kg)	12.4	14.7	8.4	6.7	—	—	8.9	—
	12.7	9.6	20.4	9.7	—	—	14.5	—
				Sawlog Lake				
No. planted/ha	—	254	254	254	254	—	—	—
	—	—	126	126	126	126	—	—
Wt planted (kg)	—	13.2	16.8	17.7	14.5	—	—	—
	—	—	14.5	24.1	25.9	21.3	—	—
Wt caught (kg)	—	9.2	3.4	3.5	1.5	—	—	—
	—	—	7.3	4.8	10.4	4.3	—	—

the growth of the same year-class planted as yearlings in the spring of 1964. Growth of the fingerlings during the winter was apparently appreciable because they were larger by July–September 1964 than their spring-planted counterparts in all lakes.

Yearlings and fall fingerlings grew rapidly in Myra and Little Mykiss lakes, habitats in which competition with other species was minimal. For example, fall fingerlings planted in Myra and Little Mykiss lakes grew to lengths of 31.0 and 27.0 cm, respectively, by the following August, whereas similar plantings in Major and Crevice lakes reached lengths of only 23.5 and 21.8 cm, respectively (Fig. 2).

Increasing the planting rate of yearling brook trout in Myra and Little Mykiss lakes resulted in a significant increase in the weight of trout recovery. The weight of trout recovered was plotted against the number of yearlings planted and proved linear. The correlation in Myra Lake ($r = 0.735$) was significant at the 10% level and in Little Mykiss Lake ($r = 0.931$) at the 1% level. No similarly obvious correlation existed between the number of fall fingerlings planted and the weight of trout recovered. There was, however, in Myra Lake, and to a lesser extent in Little Mykiss Lake, a high degree of interaction between fall fingerling survival and the following spring yearling survival in terms of weight caught (Table 2). No such interaction is apparent for the returns from the other three lakes where competition from other species was presumably high.

TABLE 3. Yields by angling and gillnetting of planted salmonids and white suckers from the study lakes.

Year	Wt[a] planted (kg)	Effort		Catch		Yield (kg/ha)		
		Angling (h/ha)	Gillnetting length of net (m)[b]	Angling (kg)	Gillnetting (kg)	Salmonids Total	Salmonids Net	White sucker total
				Myra Lake				
1963	6.4	—	915	—	26.3	4.8	3.6	
1964	7.7	—	549	—	81.7	14.9	13.5	
1965	15.4	—	1265	—	30.4	5.5	2.7	
1966	12.7	—	549	—	29.1	5.3	3.0	
1967	21.3	—	823	—	44.5	8.1	4.2	
1968	35.0	—	1159	—	52.7	9.6	3.2	
					Mean	8.0	5.0	
				Little Mykiss Lake				
1963	20.0	—	518	—	53.1	5.7	3.5	
1964	14.1	—	366	—	48.1	5.2	3.7	
1965	18.6	—	381	—	49.5	5.3	3.3	
1966	24.1	—	335	—	51.3	5.5	2.9	
1967	10.9	—	549	—	52.7	5.7	4.5	
1968	54.5	—	793	—	41.3	4.4	−1.4	
1969	2.3	—	732	—	29.5	3.2	2.9	
					Mean	5.0	2.8	
				Crevice Lake				
1962	24.1	13.9	885	19.1	15.0	1.3[c]	0.4	6.5
1963	47.7	30.2	730	11.4	14.5	2.2	0.4	4.4
1964	56.3	35.6	550	38.6	10.9	3.4	1.1	2.6
1965	104.9	54.6	490	45.4	5.9	4.4	0.2	2.8
1966	89.4	28.2	775	11.4	11.4	2.1	−1.3	4.5
1967	67.2	46.5	855	30.0	8.2	2.6	0	4.1
1968	130.3	29.3	1115	23.1	22.7	2.5	−2.6	2.1
					Mean	2.6	−0.3	3.9
				Major Lake				
1962	35.4	—	1130	—	29.1	1.1[c]	−0.2	2.8
1963	35.4	11.0	930	10.9	21.0	1.2	−0.1	3.8
1964	50.4	11.6	900	18.1	32.4	1.9	0	3.7
1965	97.6	20.6	745	17.7	7.6	1.1	−2.7	1.6
1966	—	22.7	1080	16.9	2.7	1.8	1.8	3.1
1967	—	17.8	870	1.5	3.5	1.0	1.0	4.0
1968	123.5	28.7	930	4.5	13.3	1.9	−2.8	5.0
1969	—	17.6	975	5.0	6.7	1.2	1.2	3.1
					Mean	1.4	−0.2	3.4
				Sawlog Lake				
1964	27.7	38.7	244	6.9	1.0	1.3	−3.1	6.0
1965	40.8	56.3	152	18.3	1.2	3.1	−3.4	1.4
1966	43.5	30.6	244	7.4	1.3	1.4	−5.5	1.9
1967	35.8	27.0	823	9.5	2.6	1.9	−3.8	5.5
					Mean	1.9	−4.0	3.7

[a]Includes yearlings planted in year n and fall fingerlings in year $n - 1$.
[b]Converted metric equivalent.
[c]Yields from Crevice and Major lakes include catches of planted splake. This group is not otherwise considered in this paper.

YIELDS

A mean harvest of 8.0 $kg \cdot ha^{-1} \cdot yr^{1-}$ was removed by gillnetting from Myra Lake during 1963–68 (Table 3). Similarly, Little Mykiss Lake yielded 5.0 $kg \cdot ha^{-1} \cdot yr^{-1}$. The harvest of planted salmonids from Crevice, Major, and Sawlog lakes, which was removed by angling and gillnetting, ranged between 2.6 and 1.5 $kg \cdot ha^{-1} \cdot yr^{-1}$. In addition, a mean harvest of 3.4–3.9 $kg \cdot ha^{-1} \cdot yr^{-1}$ of white suckers was removed by gillnetting from the three last-mentioned lakes.

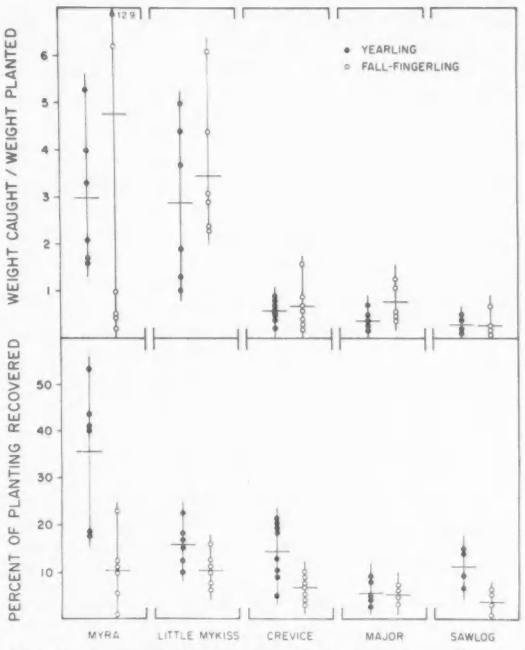

FIG. 1. Recoveries from annual plantings of yearling and fall-fingerling brook trout (*Salvelinus fontinalis*) in five study lakes; and ratios of weight recovered to weight planted. Horizontal lines represent the means.

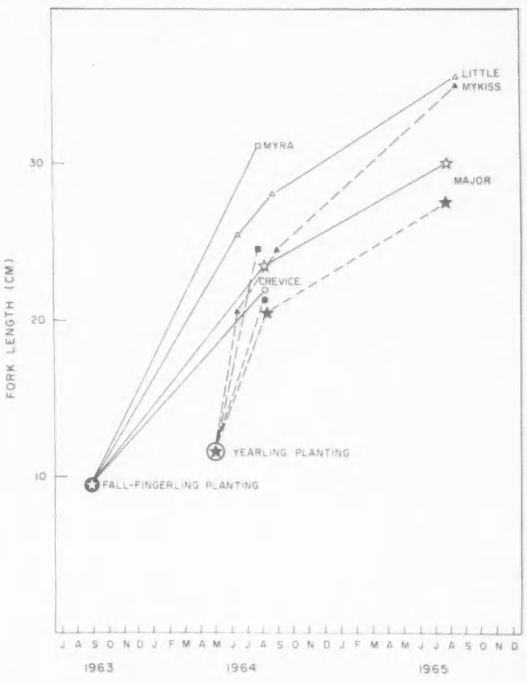

FIG. 2. Growth in length of fall-fingerling and yearling brook trout (*S. fontinalis*) from time of planting to time of recovery. Open symbols represent fall fingerlings, solid symbols represent yearlings. Numbers of fish measured were as follows: Myra, 200; Little Mykiss, 195; Crevice, 48; Major, 48.

My population estimates on the two lakes suggest that the above yields approximate maximum returns from the plantings. Numbers of brook trout extant in Myra Lake in August of the years 1963, 1967, and 1968 were estimated by the Leslie method (Ricker 1958) to be 159, 202, and 281, respectively. The annual gillnetting removed 99, 76, and 96% from these populations. Presumably the intensive gillnetting in Little Mykiss Lake removed a similarly high percentage of its population.

Trout were removed fom Crevice, Major, and Sawlog lakes by angling throughout the season and by supplementary gillnetting in August. In Crevice Lake and presumably in the others, natural and fishing mortality reduced the plantings to a low level by fall. This is substantiated by mark-and-recapture estimates of the trout populations in Crevice Lake in 1963–66 (Fraser 1972), which showed that between 91 and 288 brook trout were extant in the fall in those years.

When the weights of the plantings were subtracted from total salmonid yields, the resulting net yields ranged from a high of 5.0 $kg \cdot ha^{-1} \cdot yr^{-1}$ in Myra Lake to $-4.0\ kg \cdot ha^{-1} \cdot yr^{-1}$ in Sawlog Lake (Table 3). The poor net yield from Sawlog Lake was due in part to an excessive planting rate of 126 yearlings and 254 fall fingerlings $\cdot ha^{-1} \cdot yr^{-1}$. In only two lakes, Myra and Little Mykiss, did the harvest of trout exceed the weight of trout planted.

Discussion

This study shows that the recovery of yearlings and fall fingerlings is dependent on whether or not competing fish species are resident in the lake planted. The weight caught to weight planted ratios (Fig. 1) illustrate the difference between the two types of lakes. Myra and Little Mykiss lakes, in which there was little competition from resident fish, yielded 3 kg for each kilogram of yearling trout planted. Fall fingerling plantings were even more successful, returning 4.8 and 3.5 kg, respectively, for each kilogram planted. In contrast, the return from the three lakes supporting suckers, minnows, and sticklebacks was less than the weight planted. Resident species apparently utilized the bulk of available food, leaving little for planted trout. Consequently, only 4–7% of fingerlings planted in the fall survived to be caught by angling or gillnetting in the following summer. The mean recovery of yearlings was also low (6–15%) and apparently due to competition with resident species. These fish, especially in the earlier years of the study, had not quite reached angling size at time of planting, and although they rapidly reached catchable size (Fig. 2) their numbers were drastically re-

duced by natural mortality in the interim. In Crevice Lake, for example, the average recovery of yearlings during 1962–64, when the planted fish were small (11.9 cm), was 9%, whereas it was 18% for the larger (13.7–16.5 cm) fish planted in 1965–68.

The above recovery rates are similar to some noted elsewhere. Smith (1952b) reported negligible returns of fall fingerlings planted in New Brunswick lakes containing resident competitive species. He noted that even planting yearling trout of angling size only gave reasonable returns (8–26%) if the fish were angled during the year of planting or early the following year.

Needham and Sumner (1941) recovered, by angling, 4.3% of fall fingerlings and 25.6% of yearling brook trout planted in Upper Angora Lake, California. Wales (1946) reported 0.4–3.2% recoveries of fall-fingerling brook trout compared with a 40% recovery of planted yearlings from Castle Lake, California. In both studies the low recovery of fall fingerlings was attributed primarily to predation by large trout: brown trout (*Salmo trutta*) in Upper Angora Lake, and brown, lake (*Salvelinus namaycush*), and rainbow trout (*Salmo gairdneri*) in Castle Lake.

Predation by large trout was not likely a serious mortality factor in our study, since few fish survived long enough to become potential predators of planted trout. Fish-eating birds and mammals forage on the study lakes, especially during the openwater season, but we have no measure of the numbers of planted trout that are taken by these predators.

There was little or no competition from resident species in Little Mykiss and Myra lakes but the survival of planted fall fingerlings was apparently limited by a severe seasonal reduction in the amount of suitable trout habitat caused by the decrease of dissolved oxygen in the bottom waters during winter. Limnological data are not available for the years covered in this study, but water samples collected from Myra Lake on March 29, 1974 provide an example of dissolved oxygen concentrations in late winter. On this date a vertical series of water samples taken at 1-m intervals, when ice cover was 0.6-m thick, indicated 7 mg/L dissolved oxygen at 1 m, 2 mg/L at 2 m, and only trace amounts at greater depths.

Percentage recoveries of fall fingerlings varied considerably from year to year and probably reflect various degrees of oxygen depletion in Myra and Little Mykiss lakes. In Myra Lake, for example, the recovery of fall-fingerling plantings ranged from 0.8 to 23.0%. The high 23.0% recovery of the 1963 fall-fingerling planting followed a winter in which snow cover in the area was unusually light; 34% less than the 10-yr mean (from Snow Cover Data, Canada Department of Transport, Meteorological Branch, winters 1960–61 to 1969–70). Ice breakup in 1964 also occurred 2 wk earlier than normal. It is believed that the light snow cover in 1963–64 permitted a greater penetration of sunlight, which consequently enhanced the oxygen content of the water through increased photosynthesis in the phytoplankton. This is supported by evidence that above-average recoveries of the 1963 fall-fingerling plantings were also made in Little Mykiss Lake (Table 2) and in neighboring Shallnot Lake (Fraser 1968).

Management Considerations

Hatchery-reared brook trout planted as either fall fingerlings or yearlings survive poorly in lakes already supporting competitive species such as the white sucker. Although survival is only slightly higher in lakes that lack competitive species (e.g. Myra, Little Mykiss) the rapid growth of the survivors results in an excellent weight return. Fall-fingerling plantings can best utilize the productive potential in such lakes. When trout are planted at a rate of 50–75 fish/ha, total yields of 5–8 $kg \cdot ha^{-1} \cdot yr^{-1}$ can be expected.

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Paralytic Toxins in the Dinoflagellate *Gonyaulax excavata* and in Shellfish

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WHITE, A. W., AND L. MARANDA. 1978. Paralytic toxins in the dinoflagellate *Gonyaulax excavata* and in shellfish. J. Fish. Res. Board Can. 35: 397–402.

Paralytic toxins were examined quantitatively in a culture of *Gonyaulax excavata* and in shellfish by the standard mouse bioassay and by an alkaline peroxide oxidation–fluorometric assay for saxitoxin (STX). The dinoflagellate probably contains at least three toxins, including STX, which represents 3–20% of the total toxin load. During growth in culture the toxin content per cell decreased progressively; however, relative amounts of the three toxins remained roughly similar. In toxic shellfish from nature (*Mya arenaria, Mytilus edulis, Modiolus modiolus*) and in shellfish fed *G. excavata* under controlled conditions the relative amounts of toxins differed from those in *G. excavata*, suggesting toxin interconversions and interference with the chemical assay by toxins other than STX. The consistency in the relationships between bioassay and chemical assay results for clam samples was fair, although the chemical assay measured from 16 to 48% of the bioassay. The chemical assay is questionable as an indicator of total toxin content, and until further data are accumulated the mouse bioassay method is considered more reliable for measuring paralytic, *G. excavata* toxins in shellfish.

Key words: Gonyaulax excavata, dinoflagellate toxins, paralytic shellfish poisoning, saxitoxin, toxin measurement

WHITE, A. W., AND L. MARANDA. 1978. Paralytic toxins in the dinoflagellate *Gonyaulax excavata* and in shellfish. J. Fish. Res. Board Can. 35: 397–402.

Nous avons fait l'étude quantitative des toxines paralysantes dans une culture de *Gonyaulax excavata* et dans des coquillages à l'aide d'analyses biologiques ordinaires avec souris et le dosage de la saxitoxine (STX) dans des essais d'oxydation alcaline avec peroxyde et fluorométrie. Le dinoflagellé contient probablement au moins trois toxines, y compris la STX qui représente de 3 à 20% de la charge totale de toxines. Pendant la période de croissance en culture, le contenu en toxines par cellule augmente progressivement; cependant, les quantites relatives des trois toxines demeurent à peu près les mêmes. Dans des coquillages toxiques cueillis dans leur milieu naturel (*Mya arenaria, Mytilus edulis, Modiolus modiolus*) et dans des coquillages nourris à *G. excavata* dans des conditions contrôlées, les quantités relatives de toxines diffèrent de celles présentes dans *G. excavata*, ce qui donne à croire qu'il y a interconversions des toxines et interférence, dans l'analyse chimique, de toxines autres que la STX. Les relations trouvées par analyse biologique et analyse chimique dans des échantillons de coques sont assez uniformes, bien que l'analyse chimique donne des mesures différant de 16 à 48% de celles obtenues par analyse biologique. L'analyse chimique n'est pas un indicateur fiable de la teneur totale en toxines. En attendant d'accumuler plus de données, nous considérons l'analyse biologique avec souris comme étant une méthode plus sûre de determination des toxines paralysantes de *G. excavata* dans les coquillages.

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PARALYTIC shellfish poisoning (PSP) on both coasts of North America is caused by toxic marine dinoflagellates of the genus *Gonyaulax*. Shellfish-monitoring programs in Canada, and in other parts of the world where PSP is a problem, employ a standard mouse bioassay test to determine toxin levels in shellfish (AOAC 1975; Bond 1975; Clem 1975; Prakash et al. 1971; Quayle 1969). This bioassay has been used successfully for many years, although it has the disadvantages of not being very sensitive or accurate.

Recently several chemical assays for saxitoxin (STX), a paralytic, dinoflagellate toxin, have been developed (Bates and Rapoport 1975; Buckley et al. 1976; Gershey et al. 1977; Proctor et al. 1975). The most promising of these for routine use seems to be the alkaline peroxide oxidation–fluorometric method of Bates and Rapoport (1975), which is 100 times more sensitive than the bioassay, and which requires only small samples

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(2 g), allowing it to be used for individual animals or organs.

The chemical assay for STX has potential for shellfish monitoring on the west coast of North America, where *Gonyaulax catenella* is the major causative agent of PSP because STX seems to be the only paralytic toxin produced by this dinoflagellate (Buckley et al. 1976; Proctor et al. 1975; Schantz et al. 1975a). In the Atlantic Ocean, however, the situation is different. The organism causing PSP in eastern Canada and New England (and northeastern United Kingdom) is *G. excavata* (Loeblich and Loeblich 1975), formerly called *G. tamarensis*. This organism produces STX and several chemically similar toxins (Buckley et al. 1976; Oshima et al. 1977; Shimizu et al. 1975, 1976). STX may represent only a small percentage of the total toxin load (Oshima et al. 1977; Schantz et al. 1975a).

The usefulness of the chemical STX assay as an indicator of total paralytic toxins content in samples from the east coast depends upon the degree of consistency in the relative proportion of the toxins in the dinoflagellate and in shellfish under various conditions. Using the bioassay and two variations of the chemical assay, we examined the content of toxins in a culture of *G. excavata* during various stages of growth, in toxic shellfish from nature, and in shellfish fed *G. excavata* in the laboratory.

Materials and Methods

There are taxonomic difficulties in the "*Gonyaulax tamarensis* complex," in fact, in the entire genus (Taylor 1975; Wall 1975). The taxon *G. tamarensis* may more correctly refer to a nontoxic, nonbioluminescent organism, while the toxic dinoflagellate in eastern Canada and New England may more appropriately be referred to as *G. excavata* (Braarud) Balech (Loeblich and Loeblich 1975). In this paper we consider the toxic organism used here and in other studies (referred to as *G. tamarensis*) to be the same species, i.e. *G. excavata*.

Details regarding the isolate of *G. excavata* used and conditions of growth have been described previously (White 1976). The only difference was that the bacteria-free culture of *G. excavata* was grown in 12 L of medium in 18-L glass carboys receiving 7500 lx illumination and very gentle aeration. Cell counts were made periodically with a Model B Coulter counter and a 100-μm aperture tube. Mean cell diameter was determined by measuring the protoplast of 24 cells, chosen at random. Mean cell volume was calculated from the diameter, assuming the protoplast to be a sphere.

Soft-shell clams (*Mya arenaria*), blue mussels (*Mytilus edulis*), and horse mussels (*Modiolus modiolus*) were collected locally during the fall of 1976. The peak of the *G. excavata* bloom during the previous summer had occurred in July (White 1977). Shellfish were kept at 4–10°C for 4–24 h after collection when toxin assays were conducted.

For the feeding experiments shellfish were thoroughly brushed and cleaned, and placed in a single layer in fiberglass tanks. They were maintained in aerated, filtered seawater at 10°C in the dark for 1 wk before feeding. Mass cultures of *G. excavata* in the log phase of growth (9.9×10^5 cells/mL) were then added to the tanks. Toxin assays were conducted before feeding, 1 wk after feeding (at which time most of the dinoflagellates had been removed by the shellfish), and 2 wk after feeding.

Bioassays of paralytic toxins in shellfish were conducted as described by the AOAC (1975). The acid extracts were adjusted to pH 3.5 and sent to the Department of National Health and Welfare in Ottawa, where "10-mice" assays were conducted on each extract. Results are expressed as "micrograms STX equivalent per 100 g meat" because the bioassay is standardized with a pure STX solution.

Bioassays of toxins in *G. excavata* were made by gently collecting cells from 50 to 175 mL of culture (at least 3.3×10^5 cells total) on membrane filters (Millipore AAWP, 0.8 μm). The filter was boiled gently for 5 min in 20 mL of 0.01 N HCl. The extract was cooled, adjusted to pH 3.5, and assayed as above. Preliminary studies showed that breaking open the cells by grinding prior to boiling did not increase toxin yield. Also, the toxin yield was the same whether 0.1 N or 0.01 N HCl was used for extraction, so the latter concentration was used for *Gonyaulax* samples to minimize subsequent addition of NaOH. Using 10 replicate samples, the variability of "10-mice" assays on *Gonyaulax* extracts was 3.2%.

The chemical assays for STX were conducted according to the method described by Bates and Rapoport (1975), using the same instrumentation, except that fluorescence emission was measured at 392 nm instead of at 380 nm. This wavelength was chosen because in our samples, including STX standards, a plateau of maximum fluorescence occurred between about 390 and 398 nm. Readings at these wavelengths were generally about 10% greater than at 380 nm.

Standardization was performed by running four concentrations of purified STX solution (obtained from Dr J. E. Campbell, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio) in triplicate each day samples were assayed. Standard curves obtained in this way were found to be very nearly the same from day to day. This method of standardization was used in preference to the spectrophotometric method recommended by Bates and Rapoport (1975) because it requires much less purified STX. It was found most satisfactory to make up the 10% H_2O_2 solution freshly each day.

For the chemical assays on shellfish, 2 g of meat was taken as a subsample from the homogenized material used for the bioassay. For assays on *Gonyaulax*, 50–300 mL of culture was gently filtered (Millipore AAWP, 0.8 μm) and sucked to near dryness. The filter was folded, cut in pieces into a test tube, and 2.0 mL of 0.5 M trichloroacetic acid was added. Assays were then conducted in the normal manner. Complete and abbreviated assays were used (i.e. with and without the ion-exchange-column step, respectively).

For unknown reasons recovery of purified STX from the ion-exchange column was found to be 57.5% (with five replicates the range was 45.1–71.4%). Therefore, throughout this paper results using the column have been multiplied by a factor of 1.74 to determine total STX. STX was undetectable in 1.0 mL of 0.5 N HCl eluate taken before and after the 4.0 mL of 0.5 N HCl eluate used for testing, suggesting that the missing STX remained on the column.

Results

The doubling time of *G. excavata* in the log phase was 3.5 d (Fig. 1A). The total load of toxins, measured

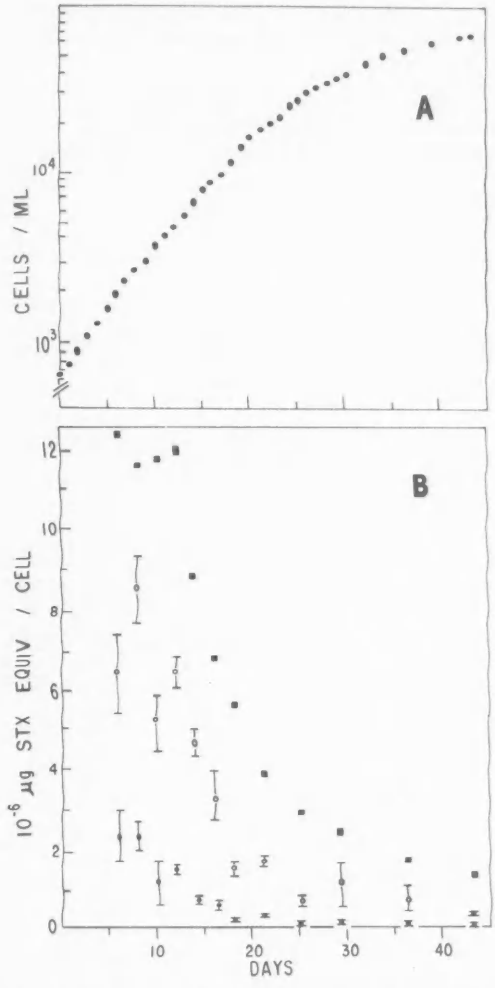

FIG. 1. Growth (A) and toxin content per cell (B) of *Gonyaulax excavata* in batch culture as measured by bioassay (■), chemical assay without the ion-exchange column (○), and chemical assay with the column (●). For chemical assays, points represent the mean of triplicate samples ($P = 0.10$).

by the bioassay, remained nearly constant at about 1.2×10^{-5} µg STX equivalent/cell during the early stage of growth (Fig. 1B). At about 4.5×10^3 cells/mL, the total toxin content began to decrease rapidly even though cells were still in the log phase of growth. Forty-three days after inoculation, during stationary phase, the total toxin content per cell had decreased to about 10% of the initial amount. As cells approached the stationary phase the mean cell volume decreased markedly (from 19 500 µm³ initially to 6200 µm³ after 43 d). However, even considered on the basis of unit cell volume, the total toxin content after 43 d was still only about 30% of the initial value.

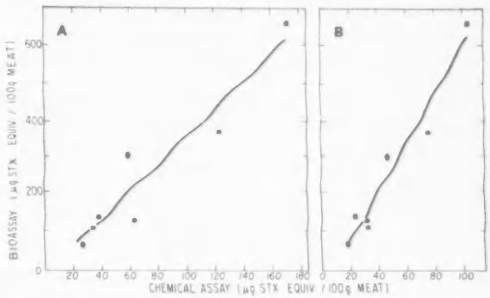

FIG. 2. Linear regressions of amount of toxins in clams from nature measured by bioassay on amount measured by chemical assays, either with the ion-exchange column (A) or without (B). For A, $y = -22.87 + 3.72x$ and $r = 0.95$; SE est. = 70.8. For B, $y = -61.72 + 6.57x$ and $r = 0.97$; SE est. = 54.3.

Results of the chemical assays for toxins, with and without the ion-exchange column, generally paralleled bioassay results during growth of the culture. However, as seen in Table 1, results with the column represented only a few percent (2.7–19.8; mean = 8.6) of the bioassay results, whereas results without the column represented a much greater percentage (25–73; mean = 45). Thus, the chemical assay without the column measured toxin(s) in addition to STX; some toxic constituent(s) of *G. excavata* is probably not measured by either variation of the chemical assay. In the chemical assays there was considerable scatter among replicates as shown by the confidence intervals ($P = 0.1$) in Fig. 1. This "within-sample" scatter obscures whether or not there is a real consistency during growth of the culture in the relationship between results of the chemical assays and the bioassay (Table 1), i.e. whether or not the amounts of the various toxins remain proportional. However, a rough parallelism in amounts is apparent from Fig. 1.

In shellfish from nature the chemical assays, with and without the column, measured fairly similar amounts for any particular sample (Table 2), unlike the situation for the *Gonyaulax* samples, although results with the column were somewhat greater than without. Generally, as a percentage of the bioassay results, the assay with the column measured more toxin in shellfish (18–48%; mean = 31) than in *Gonyaulax* (see Table 1), whereas the assay without the column measured less (14–39%; mean 23). There were, therefore, relative differences in toxin amounts between *Gonyaulax* and shellfish samples.

For shellfish, as for *Gonyaulax*, the consistency among samples in the relationship between chemical and bioassay results was fair. There were obvious positive correlations between results of the chemical assay, with and without the column, and the bioassay (Fig. 2).

Initially, after clams and mussels were maintained in filtered seawater for 1 wk following collection, toxin amounts were below the limit of detectability by the

TABLE 1. Toxins (in units of 10^{-6} μg STX equivalent/cell) in *Gonyaulax excavata* as measured by bioassay and chemical assays.

Days in culture	Bioassay	Chemical assay: With column[a]	(% of bioassay)	Chemical assay: Without column[a]	(% of bioassay)	Mean cell diam (μm)	Calculated vol (μm³)
6	12.3	2.32	(18.9)	6.38	(52)	33.4	19 510
8	11.6	2.30	(19.8)	8.51	(73)	29.9	14 000
10	11.8	1.12	(9.5)	5.21	(44)	32.1	17 320
12	12.0	1.42	(11.8)	6.46	(54)	32.1	17 320
14	8.78	0.75	(8.5)	4.67	(53)	26.1	9 310
16	6.79	0.57	(8.4)	3.32	(49)	30.4	14 710
18	5.66	0.17	(3.0)	1.47	(26)	29.7	13 720
21	3.86	0.27	(7.0)	1.70	(44)	24.0	7 240
25	2.95	0.08	(2.7)	0.75	(25)	25.7	8 890
29	2.40	0.11	(4.6)	1.14	(48)	25.8	8 990
36	1.78	0.10	(5.6)	0.79	(44)	22.9	6 290
43	1.29	0.04	(3.1)	0.40	(31)	22.8	6 200

[a]Mean of triplicate samples.

TABLE 2. Toxins (in units of μg STX equivalent/100 g meat) in shellfish from nature as measured by bioassay and chemical assays.

Organism	Date and location	Bioassay	Chemical assay: With column	(% of bioassay)	Chemical assay: Without column	(% of bioassay)
Mya arenaria	Sept. 1, 1976					
	Lepreau Basin	61	27.8	(46)	19.1	(31)
	Crow Harbour	298	59.0	(19)	46.6	(16)
	Sept. 28, 1976					
	Crow Harbour	364	124.0	(34)	76.1	(21)
	Oct. 27, 1976					
	Scotchman's Cove	142	38.3	(27)	23.8	(17)
	Letite	109	34.4	(32)	33.1	(30)
	Nov. 2, 1976					
	Lepreau Basin	133	63.3	(48)	32.2	(24)
	Crow Harbour	662	172.0	(26)	104.0	(16)
Mytilus edulis	Sept. 1, 1976					
	Lepreau Basin	147	25.7	(18)	20.0	(14)
Modiolus modiolus	Sept. 27, 1976					
	Ross Island	432	123.0	(28)	107	(25)
	Oct. 27, 1976					
	Ross Island	301	96.9	(32)	117	(39)

bioassay (i.e. <34 μg STX equivalent/100 g meat) (Table 3). Fourteen days after clams fed on *G. excavata*, the chemical assay with the column and without the column measured 22 and 12%, respectively, of the bioassay, suggesting that the proportionality of toxins in *G. excavata* changes within a few days after ingestion of the dinoflagellates by clams. In fed mussels, the chemical assay without the column showed very high unoxidized blank values, and toxin amounts measured were consequently very low, only about 2% of the bioassay.

Discussion

The results suggest that *G. excavata* contains at least three different toxins. One is STX, measured by the chemical assay using the ion-exchange column, and represents only 3–20% of the total toxin load in the dinoflagellate. One or more other toxins are measured, in addition to STX, when the chemical assay is used without the ion-exchange-column step. Finally, one or more different toxins are measured, in addition to the toxins mentioned already, only by the bioassay method. This is consistent with recent reports in which STX and

TABLE 3. Toxins (in units of μg STX equivalent/100 g meat) in shellfish fed *Gonyaulax excavata* in the laboratory.

Organism[a]	Days after feeding[b]	Bioassay	Chemical assay			
			With column	(% of bioassay)	*Without* column	(% of bioassay)
Mya arenaria	0	<34	8.5	(>25)	10.5	(>31)
	7	311	40.7	(13)	41.3	(13)
	14	135	29.2	(22)	16.5	(12)
Mytilus edulis	0	<34	23.5	(>69)	3.13	(>9.2)
	7	537	88.8	(17)	9.57	(1.8)
	14	311	60.3	(19)	5.30	(1.7)

[a]Collected from St. Andrews area on November 21, 1976.
[b]Details on feeding in Materials and Methods.

up to six other toxins chemically related to STX have been found in *G. tamarensis* (= *excavata*) and in clams (Buckley et al. 1975, 1976; Oshima et al. 1976, 1977; Shimizu et al. 1975, 1976), and in which STX was found to be a minor constituent representing only a few percent of the total toxicity (Oshima et al. 1977; Schantz et al. 1975a).

The toxin content per cell in *G. excavata* decreases progressively as the culture ages. The toxins are very likely leaked to the medium as was shown by Prakash (1967) for another strain of this organism. Our strain of *G. excavata* appears to leak toxins more readily than the strain Prakash studied; toxin content started to decrease early in the growth of the culture, whereas the decrease in Prakash's strain was not apparent until the culture had reached stationary phase. Also, maximum toxin content of these cells, as well as leakiness, may depend upon the clone examined. The maximum total toxin content here was 1.2×10^{-5} μg STX equivalent/cell, whereas in Prakash's clone it was about 3.5×10^{-5} μg STX equivalent/cell. Two new clones of *G. excavata* from the Bay of Fundy contain 4.5×10^{-5} μg STX equivalent/cell (A. W. White unpublished data).

The proportionality of the several toxic constituents of *G. excavata* seems to remain fairly constant during various stages of growth of a culture, although this is partially obscured by "within-sample" and "between-sample" variability. The relative contribution of STX to the total toxin load decreases somewhat, however, as cells enter the stationary phase of growth.

The difference in toxin proportions between *G. excavata* and shellfish samples is likely due, in part, to toxin interconversions occurring within the shellfish. There is evidence that some *G. excavata* toxins are unstable and can interconvert with themselves and with STX under certain conditions (Buckley et al. 1976; Shimizu et al. 1975, 1976). Our results suggest such interconversions may occur within a few days after ingestion of the dinoflagellate. An additional complication is that there may be interference with the chemical STX assay by the other toxins because of their close structural resemblance to STX (Bordner et al. 1975; Oshima et al. 1977; Schantz et al. 1975b; Shimizu et al. 1976).

Recently, Oshima et al. (1977) reported a similar difference in toxin proportions between *G. tamarensis* and clam samples. They found that in the dinoflagellate STX, gonyautoxin II, and neoSTX accounted for about 5, 25, and 33%, respectively, of total toxicity. Whereas in clams (*Mya arenaria*) exposed to the dinoflagellate, STX and gonyautoxin II accounted for 20 and 50%, respectively, of total toxicity; neoSTX was present only in trace amounts. These results would agree fairly well with ours quantitatively if the chemical assay with the column measures only STX and without the column measures STX plus neoSTX. This would be consistent with the view that neoSTX more closely resembles STX than do the five other gonyautoxins (Oshima et al. 1977).

Aside from the problems of toxin interconversions and interference with the STX assay by related toxins, interference with the assay by other compounds in shellfish extracts has not yet been ruled out. Bates and Rapoport (1975), in describing the chemical assay, stated that "no quenching was ever observed" using extracts from *Saxidomus giganteus* and *Mytilus californianus*. However, our results with the abbreviated assay on freshly fed *M. edulis*, which had very high blank values, suggest that some chemical interference from the extract may have occurred. In this connection, for clam samples blank values without the column were consistently greater (2.5–20 times) than those with the column, explaining the greater STX amounts for samples run with the column versus without.

Although there is a definite positive correlation between toxin measurements made by the chemical assays and the bioassay on clam samples from nature, the large standard error means that useful prediction of total toxicity in clams from results of the chemical assays may only be made at relatively high toxin concentrations. The error results, of course, from the summation of errors of the two methods. The standard bioassay uses three mice per test and has a precision of about ±20% for shellfish samples. One would expect the precision

to be better when, as here, 10 mice are used per test; it was ±3.2% for *Gonyaulax* samples. Bates and Rapoport (1975) report that the reproducibility of the chemical assay for STX is ±5%. However, in our experience with *Gonyaulax* samples it is commonly about ±20%. Some of this error may be accounted for by using the STX method in a system containing several closely related toxins.

In summary, at present the chemical assay for STX appears questionable for routine monitoring in areas where *G. excavata* is prevalent. Application of the method along the east coast depends upon a thorough knowledge of toxin interconversions and interference from toxins other than STX and from other compounds in shellfish extracts, and upon extensive field testing. For the present, despite its disadvantages, the bioassay is the more reliable method because it integrates all toxins with paralytic activity.

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Pathogenic Vibrios in Confinement-Reared and Feral Fishes of the Maine–New Hampshire Coast[1]

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Vibrio anguillarum was isolated from moribund or fresh dead confinement-reared or feral fishes from the Maine–New Hampshire coast. All *Vibrio* isolates were tested for pathogenicity by inoculation into 12–15 cm-coho salmon (*Oncorhynchus kisutch*) smolts reared in fresh water. In 1975, of 35 isolates from confinement-reared fishes, 29 were *Vibrio* and 22 of these killed test fish; 1 of 4 isolates from feral fishes was a pathogenic *Vibrio*. In 1976, of 69 isolates from cultured fishes, 52 were *Vibrio* and 12 were pathogenic; 39 of 59 isolates from feral fishes were *Vibrio*, yet only 1 was pathogenic. One group of *Vibrio* isolates, all from winter flounder (*Pseudopleuronectes americanus*), killed all salmon smolts the same day of injection; one strain (569) consistently killed smolts within 3–4 h after inoculation. With microtiter methods, agglutinin titers of rabbit antisera were determined against all pathogenic *V. anguillarum* isolates. Three distinct antigenic groups of *V. anguillarum*, confirmed by agar-gel diffusion and challenge, were found on the Maine–New Hampshire coast. Although two of these groups appear to be antigenically similar to West Coast strains 775 and 1669, a third group shows little relationship to other East Coast–West Coast serotypes.

Key words: Vibrio anguillarum, coho salmon, pathogenic *Vibrio*, confinement-reared (cultured) fishes, serotypes, agar-gel diffusion

STROUT, R. G., E. S. SAWYER, AND B. A. COUTERMARSH. 1978. Pathogenic vibrios in confinement-reared and feral fishes of the Maine–New Hampshire coast. J. Fish. Res. Board Can. 35: 403–408.

Nous avons isolé *Vibrio anguillarum* de poissons moribonds ou venant de mourir, élevés en espace restreint ou poissons sauvages capturés le long de la côte du Maine–New Hampshire. Le pouvoir pathogène de tous les isolats de *Vibrio* a été vérifié par inoculation dans des smolts de saumon coho (*Oncorhynchus kisutch*) de 12 à 15 cm élevés en eau douce. En 1975, des 35 isolats prélevés sur des poissons élevés en espace restreint, 29 étaient des *Vibrio* dont 22 firent mourir les poissons expérimentaux; 1 isolat sur 4 prélevés sur des poissons sauvages était un *Vibrio* pathogène. En 1976, sur 69 isolats prélevés sur des poissons d'élevage, 52 étaient des *Vibrio* dont 12 étaient pathogènes; 39 des 54 isolats de poissons sauvages étaient des *Vibrio*, dont seulement 1 était pathogène. Un groupe d'isolats de *Vibrio*, provenant tous de la plie rouge (*Pseudopleuronectes americanus*) causèrent la mort de tous les smolts de saumon le jour même de l'inoculation; une lignée (569) fit mourir les smolts de façon uniforme en moins de 3 à 4 h après inoculation. Nous avons déterminé par microtitration les titres d'agglutinine d'antisérums de lapin contre tous les isolats pathogènes de *V. anguillarum*. Nous avons trouvé sur la côte du Maine–New Hampshire trois groupes de *V. anguillarum* à antigènes distincts, qui ont été confirmés

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par diffusion du gel d'agar et challenge. Quoique deux de ces groupes paraissent ressembler par leurs antigènes aux lignées 775 et 1669 de la côte occidentale, un troisième groupe ne montre que peu de relation avec les autres sérotypes des côtes orientale et occidentale.

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VIBRIOSIS, a marine bacterial disease, causes severe losses among fishes held in seawater pens and impoundments (Fryer et al. 1972). The etiological agent, generally recognized as *Vibrio anguillarum*, was described by Evelyn (1971). Since finfish mariculture began in Maine and New Hampshire in 1972, vibriosis has been a major problem, causing mortality and growth suppression in confinement-reared fishes (Sawyer and Strout 1977). Immunization with an archetype *V. anguillarum* (strain 775), isolated on the U.S. West Coast, was effective in preventing the disease in coho salmon (*Oncorhynchus kisutch*) reared there (Rohovec et al. 1974), and we had similar success immunizing coho salmon here on the East Coast using a bacterin prepared from a local strain (775A) typical of the archetype organism (Sawyer and Strout 1977). The discovery of a second pathogenic vibrio (*Vibrio* sp., 1669) from Puget Sound (Harrell et al. 1976) prompted us to investigate the possibility of other pathogenic vibrios from both cultured and feral fishes in the northeast coastal area. Our objective was to determine how many vibrio serotypes were present, their prevalence, and which should be incorporated into a bacterin to protect cultured fishes on the U.S. northern East Coast from vibriosis.

Materials and Methods

SOURCE OF ISOLATES

Over a 2-yr period we sampled moribund or recently dead fishes from mariculture projects along the Maine–New Hampshire coast. The specimens, selected from groups showing increased mortality or signs of the disease, usually had some degree of hemorrhage in the fins, eyes, ventral surface, or in the body cavity. Since feral fishes may serve occasionally as food for cultured fishes, we also sampled moribund fishes of several species found near mariculture sites, as well as fishes (mostly winter flounder, *Pseudopleuronectes americanus*) caught in seines, trawls, or on the intake screens of a power plant. Some of these specimens appeared normal, while others had subcutaneous hemorrhages, possibly caused by injury at capture. Tail and fin rot were common in the flounder.

Samples of kidney tissue were streaked on Trypticase soy agar (Baltimore Biological Laboratory) with 1.5% NaCl added, and incubated at 20°C. Isolates obtained at different times or places were given sequential numbers. Except for strain 775A, those isolates taken at the same time and place from different fish were given the same number but a different letter. All colonies isolated were checked for identity as *Vibrio*; if negative, no further taxonomic clarification was made.

CHARACTERIZATION TESTS

Morphological and biochemical characteristics of the isolates were determined by the methods of Shotts and Bullock (1976). Gram stain, oxidase test, morphology and motility, fermentation or oxidation of glucose, and sensitivity to the vibriostatic agent 0/129 (Accurate Chemical and Scientific Corp., Hicksville, N.Y.) and novobiocin (Difco) at 10 μg/mL were used to identify the genus *Vibrio*. Tests for arginine hydrolysis, lysine decarboxylase, and indole production were also performed.

At this point we arbitrarily selected as a criterion for further study the ability of the *Vibrio* isolates to kill test fish upon injection. Additional biochemical tests selected by Harrell et al. (1976) were conducted on all pathogenic isolates to compare them with the archetype organism and the West Coast vibrio found in Puget Sound.

PATHOGENICITY

All isolates identified in the genus *Vibrio* were tested for pathogenicity using 12–15-cm coho salmon reared in spring water (five fish for each isolate). Replicate tests were conducted in fresh water at 15°C in 38-L aquaria containing filter and air stones. We injected each fish in the dorsal sinus with 0.1 mL of a 0.85% saline suspension containing approximately 1–2.5 × 10^7 viable bacteria. Control fish were injected with saline only. We recorded time from injection until death of each fish. Only those isolates that killed two or more of the five fish in 96 h and could be recovered in pure culture were considered pathogenic. We did not determine the LD50 of each pathogenic isolate.

SEROLOGICAL TESTS

Initially we separated pathogenic isolates into four different antigenic groups on the basis of rapid slide agglutination tests. The rabbit antisera for these tests were prepared by intravenous injection of heat-killed whole bacterial cells. Cultures were grown on Trypticase soy agar (BBL) with 1.5% NaCl for 48 h at room temperature. Then the cells were collected in 0.85% NaCl, washed once, heat killed at 100°C for 60 min, washed again and stored in 0.3% formal saline at a concentration of 10^9 cells/mL (20% *T* at 570 nm, Bausch and Lomb Spectronic 20). Rabbits were injected twice weekly in a lateral ear vein. The initial injection of 0.25 mL was increased by 0.25-mL increments at weekly intervals until the rabbit had received two 1-mL doses. After a 3-wk rest, an additional 1 mL was injected and the rabbit bled 4 d later if the titer was sufficient. We then determined agglutinin titers with the microtiter system (Cooke Engineering Co., Alexandria, Va.) using rabbit antisera and antigens prepared from each isolate. The highest serum dilution causing agglutination was recorded as titer. Each test was run at least twice and in all instances the results agreed exactly or within one dilution.

Gel diffusion (Ouchterlony) was done in 60 × 15 mm plastic petri dishes containing 1% Noble agar (Difco), 0.85% NaCl, 0.25% sodium azide, and 0.01% trypan blue in borate buffer. The antigens–antibodies reacted at 20°C for 48 h. The plates were then left at 4°C for 4 d until the precipitin lines had fully developed.

TABLE 1. Pathogenic *Vibrio anguillarum* isolates from cultured and feral fishes of the Maine–New Hampshire coast, 1973–76.

Isolate no.	Host species	Location	Date	Temp (°C)	Salinity	Circumstances
775A	*Oncorhynchus kisutch*	Wiscasset, Maine	July 1973	16	24	High mortality in net pens at commercial farm
500	*Gasterosteus aculeatus*	Biddeford, Maine	June 1975	20	31	Fish taken with beach seine
507, 508, 509	*O. kisutch*	Portsmouth, N.H.	July 1975	16	30	High mortality at UNH net pens
507, 512, 521, 521A, 522A, 522C, 523B, 528, 531A, 531B, 532, 533, 534, 535, 540A, 543A, 547A, 547B	*O. kisutch*	Wiscasset, Maine	July–Aug. 1975	16–19	24–28	Moderate to high mortality in net pens at a commercial farm
569	*Pseudopleuronectes americanus*	Durham, N.H.	Oct. 1975	9	25	Moderate mortality in holding tanks (flow-through seawater system) at Jackson Estuarine Lab
105A	*Microgadus tomcod*	Wiscasset, Maine	July 1976	19	22	Fish caught on intake screens at a power plant
630B, 650A	*Liopsetta putnami*	Durham, N.H.	July 1976	16–18	20–22	Moderate mortality in holding tanks at Jackson Estuarine Lab
630C, 630D, 632A, 633A, 634A, 635C, 635D, 637A, 638A	*P. americanus*	Durham, N.H.	July–Aug. 1976	18–20	20–22	Moderate mortality in holding tanks at Jackson Estuarine Lab
653B	*Salmo gairdneri*	Vinalhaven, Maine	Aug. 1976	16	31	Low to moderate mortality in net pens at commercial farm

Results

ISOLATES

A sample from the kidney of a moribund confinement-reared fish generally produced a pure culture of vibrio, but similar samples from feral fishes often yielded vibrio and nonvibrio isolates.

Of 35 isolates obtained from cultured fishes in 1975, 29 were assigned to the genus *Vibrio* and 22 of these killed test fish. One of the four isolates from feral fishes was a *Vibrio* which proved to be pathogenic to coho smolts. In 1976 we made 69 isolates from cultured fishes; 52 were vibrios and 12 of these were pathogenic. Of 59 isolates from feral fishes 39 were vibrios, yet only 1 was pathogenic. From the total of 81 *Vibrio* isolates from confinement-reared salmonids, 42% were pathogenic compared with only 5% of the vibrios recovered from feral fishes. Table 1 lists the pathogenic *Vibrio* isolates. As isolates 630 B, C, and D were taken from flounders held in tanks for 4 d after capture, we could not determine whether the infection was acquired in the wild or during the holding period.

CHARACTERIZATION TEST

All the pathogenic Maine–New Hampshire vibrios reacted similarly to selected biochemical tests and appear to be *V. anguillarum*, as they conform to the archetype organism and to the *V. anguillarum* (775) found on the West Coast (Table 2). Although the biochemical pattern characterized all pathogens, many isolates with this pattern were not pathogenic in salmon smolts at the temperature we used. Nonpathogenicity was predicted consistently by negative arginine and positive lysine tests.

PATHOGENICITY

We placed pathogenic isolates into two groups on the basis of the time required to kill test fish. Group 1 isolates (569, 632A, 633A, 634A, 638A, all from winter flounder) killed all five fish the same day of injection; one of these isolates (569) consistently killed fish within 3–4 h after inoculation. The injected fish showed few if any signs of disease before death. Group 2 isolates killed at least 40% of the test fish in 24–96 h; these fish usually showed external or internal hemorrhage.

SEROLOGY

Antigens of strains 775A and 569 are similar to the West Coast 775 and 1669, respectively (Table 3). Three

TABLE 2. Selected properties of pathogenic *Vibrio anguillarum* isolates from Maine–New Hampshire compared with those from Puget Sound and with the archetype *V. anguillarum*.

	All Maine–New Hampshire *V. anguillarum*	Puget Sound *V. anguillarum* 775 (Harrell et al. 1976)	Puget Sound *Vibrio* sp. 1669 (Harrell et al. 1976)	Archetype *V. anguillarum* (Evelyn 1971)
Gram reaction	−	−	−	−
Motility	+	+	+	+
Oxidase test	+	+	+	+
Sensitive to 0/129	+	+	+	+
Acid from glucose	+	+	+	+
Gas from glucose	−	−	−	−
Arginine hydrolysis	+	+	−	+
Lysine decarboxylase	−	−	−	−
Indole production	+	−	−	+
Voges–Proskauer	+	+	−	+
Acid from				
Arabinose	+	+	−	+
Glycerol	+	+	−	+
Mannitol	+	+	+	+
Sucrose	+	+	+	+
Galactose	+	+	−	+

of the four East Coast isolates (775A, 569, 507) used to produce rabbit antisera appear serologically distinct, as each shows little titer against the other antisera. Although we did not detect agglutination between strain 508 and heterologous antisera in rapid slide agglutination tests, microtiter results show that it is serologically similar to 569 and the West Coast 1669.

The results of the gel-diffusion tests confirmed the antigenic differences detected by microtiter agglutination. When the three antigens were tested against antiserum to 569, the only precipitin band that formed was by 569 against its homologous antiserum; no reactions were detected with either 775A or 507. When 775A antiserum was used, a strong precipitin band formed between it and 775A antigen, with a less distinct band adjacent to antigen 507. When 507 antiserum was used, a strong band was clearly visible between homologous antigen and antiserum, but there was also a weak band of partial identity with 775A, indicating the antigens are related but not identical.

Discussion

Because of the importance of the control of vibriosis to the success of rearing salmonids in confinement along the Maine–New Hampshire coast, and because the backbone of this control must be based on preventive medicine and immunization, we attempted to determine how many antigenic types of *V. anguillarum* appeared to be involved in disease outbreaks of confinement-reared fishes, and which types predominated. It is also important to determine the presence of this pathogen in feral fishes and their possible role as reservoirs of the organism along the coast.

Our results verified that pathogenic *V. anguillarum* occurs in confined and feral fishes. The presence of this organism in wild stocks may serve as a constant reservoir of infection for impounded as well as feral fishes, possibly resulting in an adverse impact on local fish populations as well as on stocking programs involving anadromous species. The importance of this possibility is emphasized by the extreme virulence of group 1 isolates in coho salmon. These strains, all antigenically similar to 569, were recovered from winter flounder and consistently killed smolts as rapidly as 3 h in some instances and always within 12 h following inoculation of 1–2.5 $\times$ 10^7 organisms at 15°C. We have not studied the mechanism of such an acute response, and we are not aware of any other reports of similar virulence by any strains of *V. anguillarum* in any host.

For this study we arbitrarily selected only those strains of *V. anguillarum* that on inoculation killed coho smolts in 96 h or less. According to this interpretation of pathogenicity, it is significant that all local pathogenic isolates were arginine hydrolysis positive and lysine decarboxylase negative, confirming Shewan's work (1963), while nonpathogenic isolates did not conform to this pattern. This group of nonpathogens was common in wild winter flounders and the fact that we could not prove them pathogenic is probably less important than the high incidence of the organisms in wild stocks. Also, we recognize that nonpathogenic strains of *Vibrio* may be good antigens against pathogenic organisms.

All pathogenic *V. anguillarum* strains showed an antiserum titer of 1:160 or more with at least one of the three serotypes (775A, 569, 507). The most common antigen was that present in strain 775A, which reacted significantly with 26 of the 37 strains. Of those 26 reactants, 19 had titers within two dilutions of the

TABLE 3. Agglutinin titers of rabbit antisera against Maine–New Hampshire strains of pathogenic *Vibrio anguillarum* using rabbit antisera.

Isolates	Antisera					
	East Coast — Maine and New Hampshire				West Coast — Puget Sound, Wash.	
	775A (homologous titer 640)	569 (homologous titer 160)	508 (homologous titer 5120)	507 (homologous titer 640)	1669 (homologous titer 1280)	775[a] (homologous titer unknown)
775A Group						
775A	640	10	10	40	10	1280
509	320	20	20	80	<10	160
512	160	20	10	20	10	160
521	160	10	10	40	10	80
521A	320	20	10	80	20	160
522A	320	20	<10	40	<10	1280
522C	320	10	10	20	10	40
523B	320	10	10	80	20	40
527	320	10	10	80	10	1280
528	320	20	10	10	10	160
531A	160	10	10	20	10	1280
531B	160	20	10	20	10	80
533	160	20	10	20	10	80
534	160	20	40	20	10	1280
540A	160	10	10	20	<10	640
547A	160	10	10	40	10	80
569 Group						
569	40	160	640	20	160	20
105A	<10	640	5120	10	320	—
508	20	320	5120	10	160	10
630B	10	640	1280	<10	160	—
630C	80	160	2560	<10	640	—
630D	10	640	1280	<10	320	—
632A	10	640	1280	<10	160	—
633A	10	640	2560	<10	160	—
634A	20	320	5120	20	640	—
635C	20	1280	2560	20	320	—
635D	40	1280	1280	160	640	—
637A	<10	640	1280	10	160	—
638A	<10	640	2560	10	—	—
650A	<10	640	640	10	160	—
653B	<10	640	320	10	160	—
507 Group (and intermediates)						
507	40	20	80	640	10	80
500	160	20	320	320	10	320
532	640	10	10	320	20	160
535	160	20	10	160	<10	320
543A	320	20	10	160	<10	640
547B	80	20	10	40	<10	80

[a]Supplied by A. J. Novotny, National Marine Fisheries Service, Manchester, Wash.

homologous titer. Antigen 569 did not induce as strong a response with rabbit antiserum but the antigenic differences between it and 775A are pronounced. Generally, strains reacting strongly against 775 were weak against 569, and vice versa. Thirteen strains reacted against 569 with titers equal to or higher than its homologous titer. Strain 508 had a very high rabbit titer (5120), but appears to be quite similar antigenically to strain 569, reacting in a parallel manner with comparable antigens. Since strain 507 differs by at least four dilutions from either 775A, 569, or 508, the justification for using it in the bacterin appears valid. An important observation is the presence of more than one antigenic type from a single outbreak.

There are three rather distinct antigenic groups of *V. anguillarum* on the Maine–New Hampshire coast

(775A, 569, 507), and although two of these groups are antigenically similar to those on the northwest coast, one group, represented by strain 507, appears to show little relationship to the other East Coast–West Coast serotypes.

Antigenic groups 775A and 569 predominated, while group 507 occurred less frequently. Although antigenic heterogeneity occurred in all three groups, group 507 showed the greatest diversity, with some strains having common antigens with the 775A isolates but essentially no association with the 569 group.

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Polarographic Studies on the Nature of Cadmium in Scallop, Oyster, and Lobster[1]

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CHOU, C. L., J. F. UTHE, AND E. G. ZOOK. 1978. Polarographic studies on the nature of cadmium in scallop, oyster, and lobster. J. Fish. Res. Board Can. 3: 409–413.

Free and bound forms of cadmium were determined in raw shellfish by use of differential pulse polarography and atomic absorption spectrophotometry. Free cadmium is defined by its polarographic peak potential of -0.62 ± 0.02 V (saturated calomel electrode) in solvent washed ammonium sulfate extracts. Bound cadmium was determined by subtracting the free cadmium from the total cadmium present in the meat. Both scallop (various species) and American lobster (*Homarus americanus*) muscle tissues contain no free cadmium. Oyster (various species), on the other hand, had a considerable percentage ($\sim 50\%$) of its total cadmium present as free cadmium, a phenomena as yet unexplained. The detection limit for free cadmium is approximately 0.05 μg/g raw tissue.

Key words: cadmium, polarography; free cadmium, oyster, lobster, scallop

CHOU, C. L., J. F. UTHE, AND E. G. ZOOK. 1978. Polarographic studies on the nature of cadmium in scallop, oyster, and lobster. J. Fish. Res. Board Can. 3: 409–413.

Nous avons dosé par polarographie à impulsions différentielles et spectrométrie d'absorption atomique les formes liée et libre du cadmium dans des crustacés et mollusques crus. Le cadmium libre est défini par un potentiel polarographique de pointe de -0.62 ± 0.02 V (électrode au calomel saturé) dans un solvant d'extraits de sulfate d'ammonium lavés. Nous avons déterminé le cadmium lié en soustrayant le cadmium libre du cadmium total présent dans la chair. Les tissus musculaires du pétoncle (diverses espèces) et du homard d'Amérique ne contiennent pas de cadmium libre. Par ailleurs, une portion considérable ($\sim 50\%$) du cadmium total présent dans l'huître (diverses espèces) est libre, phénomène inexpliqué jusqu'à maintenant. La limite de détection du cadmium libre est approximativement 0.05 μg/g de tissu cru.

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THE presence of cadmium in biological tissues, often bound to specific proteins, has drawn much attention. The isolation and characterization of these cadmium-binding proteins (metallothioneins) from various species by gel filtration have been reported (Casterline and Yip 1975; Cherian 1974; Margoshes and Vallee 1957; Nordberg et al. 1971; Piscator 1964; Shaikh and Lucis 1971; Singh et al. 1972; Weser et al. 1973) and the existence of free Cd^{2+} in certain biological material has also been suggested (Casterline and Yip 1975; Coombs 1974). The toxicological differences between metallothionein-bound Cd and unbound Cd are complex and not fully understood (Webb 1975; Nordberg and Nordberg 1975).

The accumulation of cadmium by shellfish has been well documented. Unusually high total cadmium levels have been reported — 2.40 μg/g in American oyster (*Crassostrea virginica*) (Shuster and Pringle 1969), 5.75 μg/g in New Zealand Dredge oyster (*Ostrea lutaria*) (Nielsen 1975), and from 2.82 to 16.73 μg/g in American lobster (*Homarus americanus*) digestive gland depending upon catch location (Freeman and Uthe 1974). In light of the levels found in shellfish and the observed toxicological differences, methodology for determining the free Cd^{2+} and bound Cd^{2+} in shellfish is critical. This paper describes a new polarographic method for determining free Cd^{2+} in shellfish tissue and reports levels of free and bound Cd^{2+} in three types of shellfish.

The use of a polarographic technique for measuring free Cd^{2+} is based on (a) soluble free Cd^{2+} can be determined selectively through control of supporting electrolytes and pH without completely separating all potential interferences (these interferences may not be acceptable with techniques such as spectrophotometry), and (b) information of the types of Cd-binding substances in tissue may also be obtained because the electrode responds differently to different complexes of Cd.

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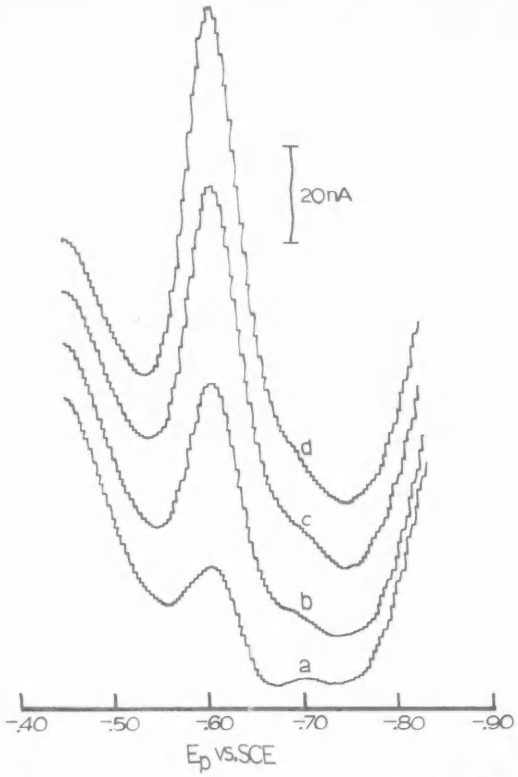

FIG. 1. Polarogram of fresh oyster sample in 1 M $(NH_4)_2SO_4$ after chloroform–hexane–chloroform washing. Stock Cd solution was used to give (a) 0, (b) 25, (c) 50, (d) 75 ng/mL added Cd^{2+} concentration to the polarographic analyte.

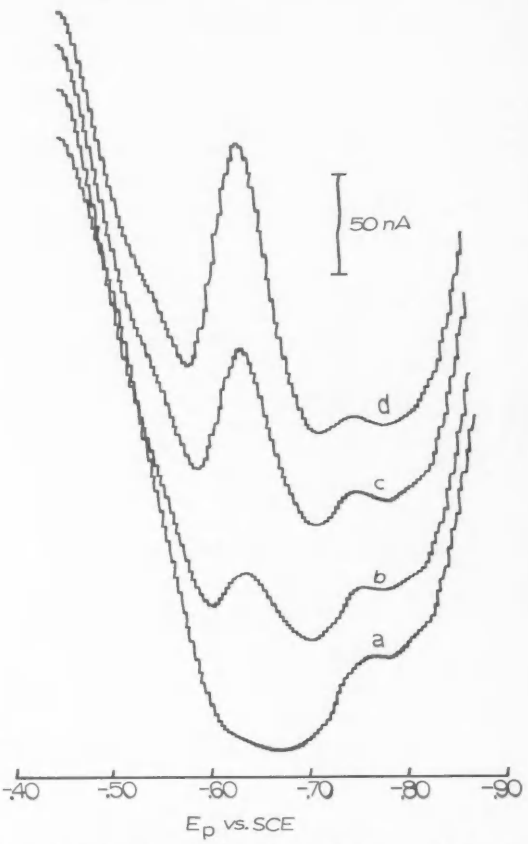

FIG. 2. Polarogram of fresh lobster meat sample in 1 M $(NH_4)_2SO_4$ after chloroform–hexane–chloroform washing. Stock Cd solution was used to give (a) 0, (b) 50, (c) 100, (d) 150 ng/mL added Cd^{2+} concentration to the polarographic analyte.

Methods

A 1-M $(NH_4)_2SO_4$ solution (Ultrapure grade, Mann Research Labs., USA) was prepared and adjusted to pH 7.0 with 1 M NH_4OH. Chloroform (ACS grade, J. T. Baker Chemical Co.) was redistilled before use. "Distilled in glass" hexane (Caledon Laboratories Ltd., Canada) was used. The 10-μg/mL cadmium standard solution was prepared weekly from commercially available $CdCl_2$ stock solution (1000 μg/mL) (Harleco, USA) by dilution with 1 M $(NH_4)_2SO_4$, pH 7.0.

A Princeton Applied Research Corporation (PAR) model 174 Polarographic Analyzer equipped with a 172 Drop Timer and an Omnigraphic Model 2000 Recorder (Houston Instrument) was used throughout. A differential pulse operating mode, a potential scan rate of 2 mV/s, a drop time of 2 s, and a modulation amplitude of 50 mV were used. Mercury was cleaned with diluted HNO_3 and rinsed with doubly distilled water. All the measurements were carried out in a cell with a KCl bridged, saturated calomel electrode (SCE) to avoid contamination of the electrode by solutions. All potential values were referred to the SCE. The peak potential of divalent Cd in 1-M $(NH_4)_2SO_4$ electrolyte is -0.62 ± 0.02 V.

All total Cd levels of samples, extracts, washes, and precipitates were determined with a Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer fitted with a high temperature heated graphite furnace (Model HGA 74 and Controller Model 2100) using the method of standard additions following digestion with H_2SO_4/HNO_3 (4/1:vol/vol). The washed aqueous extractions were directly analyzed without acid digestion.

Sample Preparation and Extraction

Fresh edible tissues of American lobster (*Homarus americanus*) tail muscle, scallop (various species) adductor muscle, and whole shucked oyster (various species) were homogenized with a Polytron Homogenizer (Kinematica GMBH Luzern-Schweiz) and then stored at −30°C.

Homogenized tissue (0.5 g) was weighed into a 40-mL graduated, screw cap centrifuge tube; 20 mL of $(NH_4)_2SO_4$ solution and 10 mL $CHCl_3$ were added and the tube was shaken for 10–15 min on a wrist action shaker (Burrell Co., Pa., USA) fitted with extension arms to give a 6–7 cm stroke. After centrifugation (at 300 × *g* for

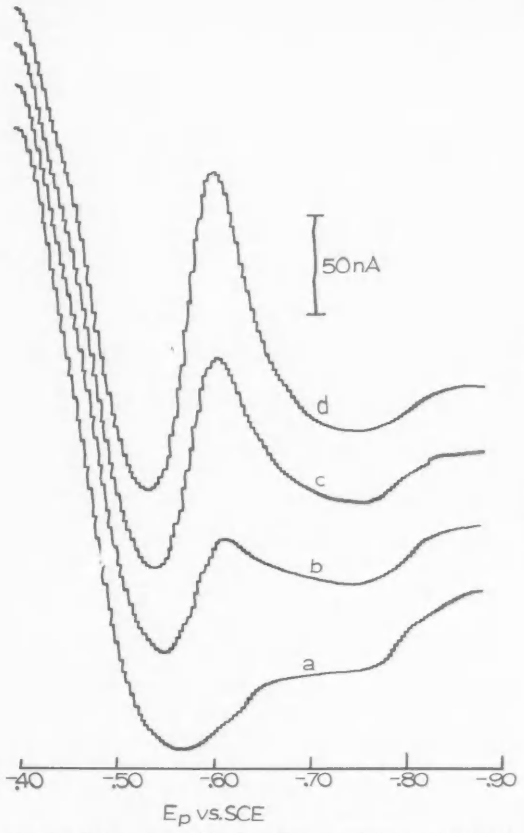

FIG. 3. Polarogram of fresh scallop sample in 1 M $(NH_4)_2SO_4$ after chloroform–hexane–chloroform washing. Stock Cd solution was used to give (a) 0, (b) 50, (c) 100, (d) 150 ng/mL added Cd^{2+} concentration to the polarographic analyte.

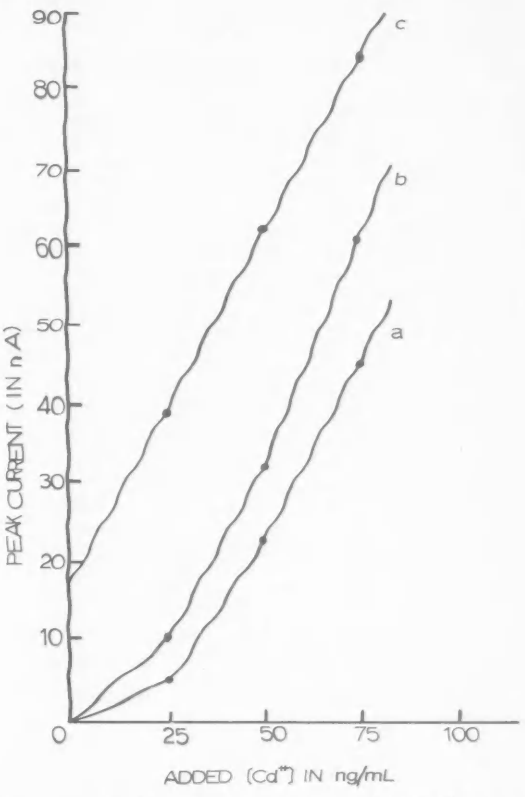

FIG. 4. The Cd peak current at -0.62 ± 0.02 V plotted against the concentration of Cd^{2+} added to the extracts of (a) lobster meat, (b) scallop, (c) oyster.

10 min) the aqueous layer was transferred with a disposable Pasteur pipette to another centrifuge tube, reextracted with fresh $CHCl_3$, and the aqueous layer washed twice with 10-mL portions of hexane. The hexane layer was separated from the aqueous layer by transferring both into a 60-mL filter tunnel (with a coarse porosity fritted disk) which passed the aqueous layer through without suction into another centrifuge tube. In this way, loss of significant amounts of the aqueous layer was avoided, and also a better separation of both layers was achieved. After the chloroform and hexane washings, the aqueous layer was washed with further 10-mL portions of chloroform until the last chloroform layer separated cleanly. A measured portion (10 or 15 mL) of

TABLE 1. Waves observed in differential pulse polarography of extracts of scallop, oyster, and lobster meat washed in 1 M $(NH_4)_2SO_4$ electrolyte (scan range -0.20 to -1.25 V).

Sample \ Peak potential (in V)	Ea_1	Ea_2	Ea_3	Ea_4	E_{Cd}
Raw scallop	−0.405[a]	−0.670[b]	−0.860[b]	−1.250[c]	—
Raw oyster	−0.435[a]	−1.037[c]	−1.159[c]	—	−0.620
Raw lobster meat	−0.395[a]	−0.770[b]	−0.887[a]	−1.135[c]	—

[a]Well-shaped wave. [b]Small broad peak < 25 nA. [c]Peak height > 2 μA.

TABLE 2. Distributions (μg/g original tissue and percent) of free Cd^{2+} and total Cd and percent recovery of added Cd^{2+} in aqueous, chloroform, and precipitate fractions from extracts of raw scallop, oyster, and lobster edible tissues. ND: none detected. N: number of run.

Sample	Total Cd	Free Cd^{2+} 1st extn.	Free Cd^{2+} 2nd extn.	Total Cd 1st extn.	Total Cd 2nd extn.	Total Cd $CHCl_3$	Total Cd PPT	Total Cd Sum	Free Cd^{2+}/ total Cd of 1st extn.	Spiked Cd^{2+} recovery %[b]
Scallop	5.32	ND	ND	0.62 (11.4%)	0.14 (2.57%)	0.02 (0.37%)	4.71 (86.6%)	5.45	0.00	—
Scallop + Cd^{2+} spiked[a]	7.28	ND	ND	0.49 (6.99%)	0.08 (1.14%)	0.00	6.47 (91.9%)	7.04	0.00	0
Oyster	0.85	0.38 (48.7%)	ND	0.50 (64.1%)	0.17 (21.8%)	0.03 (8.9%)	0.08 (10.2%)	0.78	76.7%	—
Oyster + Cd^{2+} spiked[a]	2.72	2.16 (79.1%)	ND	2.39 (87.6%)	0.18 (6.60%)	0.00	0.17 (5.8%)	2.64	91%	96.8±4.5 (N = 4)
Lobster meat	0.03	ND	ND	0.00	0.00	0.00	0.05 (100%)	0.05	0.00	—
Meat + Cd^{2+} spiked[a]	2.05	ND	ND	0.02 (1%)	0.00	0.00	2.10 (99%)	2.12	0.00	0

[a]Amount of added Cd^{2+} in sample = 2 μg/g tissue.

[b]Percent recovery = $\frac{(\text{free Cd in spiked sample} - \text{free Cd in sample})}{\text{amount of Cd spike}} \times 100$.

the aqueous layer was then transferred to a 5–50 mL PAR polarographic cell. The solution was deoxygenated by a 10-min purge with N_2 (washed with $CuCl_2$ to eliminate O_2). To eliminate any matrix effect, the divalent Cd peak was quantitated by the method of standard additions.

In recovery studies, the homogenized samples were spiked with a suitable amount of $CdCl_2$ prior to $(NH_4)_2SO_4$ extraction and the recovery of free Cd in the final extract determined.

In this study, we used ammonium sulfate because (a) it is the recommended salt for precipitating protein from biological extracts (Dixon and Webb 1961; Robinson and Grant 1966; Kagi and Vallee 1960; Cherian 1974), and (b) ultrapure ammonium sulfate is commercially available. It is possible with ammonium sulfate to remove and salt out many interferences prior to the polarographic analysis. In principle, the high purity of electrolyte reagent is required for polarographic measurement.

Organic solvents have played an important role in this study in removing other biological interferences from the ammonium sulfate extract. Initially, chloroform was used to extract most hydrophobic substances such as lipids but aqueous layers that had been washed only with chloroform foamed during N_2 purging prior to analysis and resulted in nonreproducible polarographic responses and inaccuracies in the method of standard additions, i.e. the peak current of Cd^{2+} reduction did not increase in proportion to the concentration of added Cd^{2+}. Further washing with benzene, toluene, or hexane overcame the foaming problem. Hexane, as the least toxic solvent, was selected for use throughout. Representative polarograms (Fig. 1–3) with results described in Table 1 show that each type of tissue yields its own characteristic polarogram.

Ideally, the background of a polarogram is obtained by running the electrolyte solution alone in the analyzing potential range, but this is impossible with real samples. Figures 1–3 show that the background current is dependent on each type of biological tissue. The nonlinear repression of the Cd^{2+} reduction peak current at -0.62 ± 0.02 V vs. the concentration of Cd added to the extracts of scallop and lobster is shown in Fig. 4. The binding of added Cd^{2+} into nonlabile complexes such as metallothionein is the most likely cause of the suppression observed in peak current with both scallop and lobster tissues.

With use of differential pulse polarography to determine free Cd^{2+} from biological material, the detection limit is 0.05 μg/g of fresh tissue or equivalent to around 2 μg/mL in the resultant electrolyte solution. This is somewhat dependent on the type of biological material studied, the extent of cleanup obtained, and the previous sample storage handling. Poor cleanup, resulting in masking of the Cd^{2+} peak, is the most likely cause of error in this procedure.

The presence of free Cd^{2+} (approximately 50% of the total Cd) in oysters is significant. Oysters accumulate and concentrate cadmium from sea water and must bind cadmium at least during the animal's life. Binding intracellularly would lead to accumulation but these complexes must be very labile and break down when the animal dies.

Levels of Free Cd^{2+} in Shellfish

The levels of free Cd found in the three shellfish tissues are shown in Table 2. Cadmium was tightly bound in raw scallop and lobster tissues, and no free Cd was detected;

in fact, as indicated above, added Cd^{2+} was also bound by these extracts. Free cadmium was detected only in oyster meat samples. In the first extraction of a raw oyster sample, the free Cd was approximately 76% of total Cd present in the aqueous layer. The total Cd in the extract represented 64% of the total Cd present in the tissues. This means that part of the Cd (23%) (in the extract) is bound in some sort of complex. These complexes need further investigation with other techniques. No further Cd^{2+} was found in a second extraction of the first extraction residue of oyster tissue, therefore further extraction was unnecessary. Slight amounts of the total Cd of extracts (0.37–3.9%) were extracted into the chloroform (Table 2); the chemical nature of this cadmium is unknown, but the possibility of lipid complexes should be considered.

The recoveries of cadmium added to the tissues before extractions are given in Table 2. Good recovery of Cd^{2+} was found only with oyster tissue with a single extract. This is in agreement with previous results which suggest that heavy metals such as Cd, Cu, and Zn naturally present in oyster may be unbound (Casterline and Yip 1975; Coombs 1974). The poor recoveries of added Cd demonstrated that both intact scallop and lobster muscle tissue have the potential to bind Cd tightly.

Acknowledgments

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A Simple Model For Cross-Shelf Mixing on the Scotian Shelf

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The longshore gradients of temperature, salinity, and nitrate concentration along the Scotian Shelf, and their fluxes at a fixed mooring south of Halifax, have been measured separately. Water-mass analysis indicates that the water at the eastern end of the shelf is diluted with slope water by 40% at Halifax as a result of cross-shelf mixing. Recent estimates of the annual mean transport along the shelf are used to infer the longshore gradients supported by the shelf-break fluxes in the context of a simple box model. The observed longshore gradients of temperature, salinity, and nitrate concentration are consistent with productivity requirements and measured low-frequency eddy fluxes at the edge of the Scotian Shelf.

Key words: low-frequency eddy fluxes, nutrient enrichment, longshore gradients, cross-shelf mixing, Scotian Shelf

HOUGHTON, R. W., P. C. SMITH, AND R. O. FOURNIER. 1978. A simple model for cross-shelf mixing on the Scotian Shelf. J. Fish. Res. Board Can. 35: 414–421.

Les gradients côtiers de température, salinité et concentration de nitrates le long du plateau néo-écossais et leurs fluctuations à un point fixe au sud d'Halifax ont été mesurés séparément. L'analyse de la masse d'eau indique qu'à l'extrémité orientale du plateau, l'eau est diluée par de l'eau de pente dans une proportion de 40% à Halifax, par suite d'un mélange en travers du plateau. A l'aide d'estimés récents du transport moyen annuel le long du plateau, les auteurs en déduisent les gradients côtiers, appuyés des changements causés par le plateau dans le contexte d'un simple modèle de boîte. Les gradients côtiers observés de température, salinité et concentration de nitrates s'accordent avec les exigences de la productivité et les courants tourbillonnaires de basse fréquence mesurés à la marge du plateau néo-écossais.

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THE classical explanation for the fertility of continental shelves is that land-derived nutrients are the major enriching mechanism. This concept was supported in part because available biological information suggested a general decline in biological rates and standing stocks with increasing distance from shore. Ketchum and Keen (1955) critically examined the role of runoff onto the continental shelf of the middle Atlantic states and concluded that the supply was insufficient to meet annual phytoplankton requirements. Riley (1967) expanded on Ketchum and Keen's observations and suggested that diffusion of nutrient-rich slope water onto the shelf followed by vertical enrichment of the euphotic zone could satisfy the observed nutrient requirements. Fournier et al. (1977) suggested that the transfer of nutrient-rich slope water onto the Scotian Shelf might occur via a weak advective exchange or else a series of sporadic slope water intrusions. This conclusion was based principally on the fact that the circulation over the outer portion of the Scotian Shelf is dominated by low-frequency events (e.g. Liem and Hachey 1935; McLellan et al. 1953; Hachey 1953; Warner 1970). In addition, Fournier et al. (1977) clearly showed that

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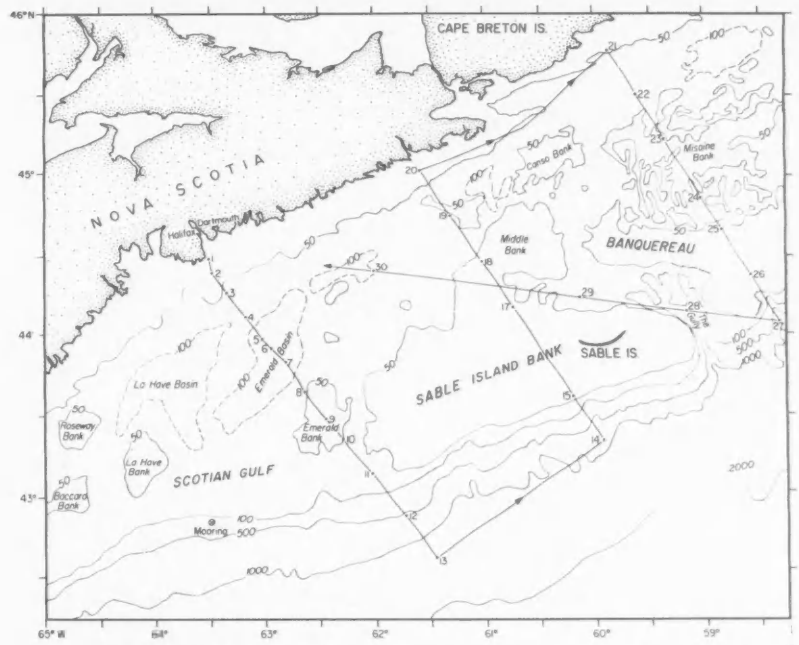


FIG. 1. Scotian Shelf showing bathymetry cruise track and mooring site. Depth contours in fathoms.

biological rates and standing stocks increase with distance from shore, reaching a maximum usually in the vicinity of the shelf break.

One assumption made by Fournier et al. (1977) in suggesting their transfer model was that longshore enrichment was negligible during the nutrient-poor summer months. This assumption was based on the fact that the outflow from the Gulf of St. Lawrence, which is a principal component of shelf water, is concentrated in the upper 50 m of the Cabot Strait (Sutcliffe et al. 1976) and thought to be nutrient poor (Steven 1974). No longshore gradients of nutrients and hydrographic properties have been measured on the shelf proper.

In this paper we examine the relative contributions of longshore and cross-shelf nutrient transport and assess the nutrient uptake model proposed by Fournier et al. (1977).

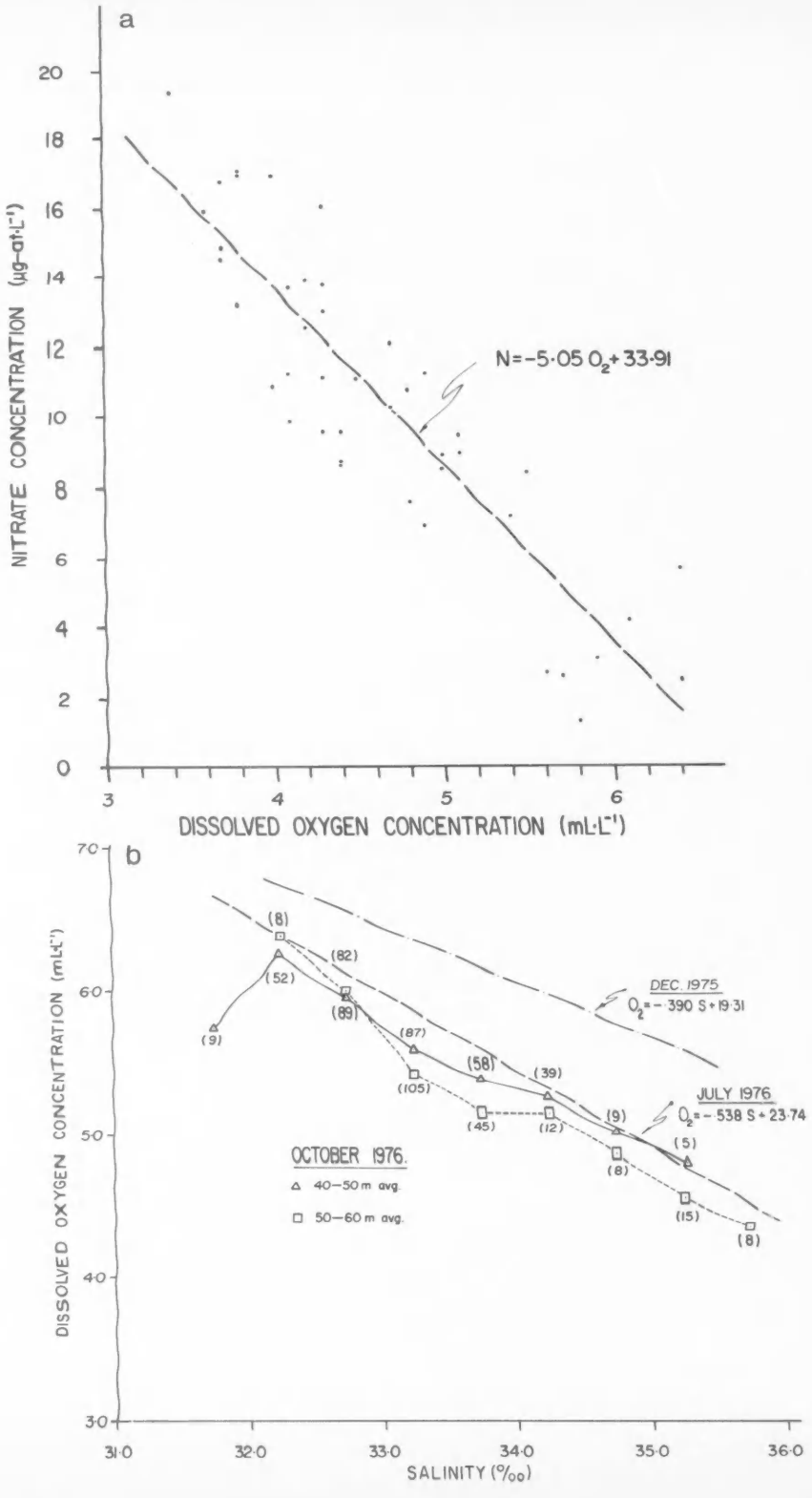
Materials and Methods

Three transects, containing a total of 29 stations, were run normal to the coast of Nova Scotia (Fig. 1), June 14–19, 1976. At all stations (except for stations 4, 5, 7, 8, and 10) the sampling routine was identical and consisted of continuous profiles of temperature and salinity plus water-bottle samples at standard depths between 0 and 300 m, depth permitting. Each sample was analyzed for nitrate and phosphate by the techniques described in Strickland and Parsons (1972).

Temperature and salinity values used in the hydrographic analysis were determined from analog X-Y recorder traces which had high-frequency noise less than 0.1°C and 0.2‰, respectively. The traces were within 0.4°C and 0.1‰ of the values recorded by reversing thermometers and bottle samples during the CTD cast. The analysis of nitrate is accurate to ± 0.1 μg-at (atoms) $N \cdot L^{-1}$.

Measurements of the shoreward fluxes of heat and salt were taken at a single mooring (Fig. 1), which was in place from December 1975 to April 1976. Aanderaa RCM-5 instruments were located at depths of 50, 150, and 230 m to measure current speed and direction, temperature, and conductivity at 20-min intervals. Since the shelf break runs east–west at this site, the currents were resolved into easterly (longshore) and northerly (onshore) components (U, V) and a salinity record was produced from those of temperature and conductivity. The spectral analysis of these data was carried out in blocks of 28.4 d with a fast Fourier transform technique, and the shoreward heat and salt fluxes were determined from the cospectra of northward velocity V with those of temperature, T, and salinity, S. For purposes of comparison with the hydrographic results on the shelf, only the 50-m results will be used, since the average depth of the Scotian Shelf is 90 m.

The technique used to determine the shoreward fluxes of nutrients, in particular nitrate, from the corresponding heat and salt transports, is outlined briefly below. (Details of the method are available from P. C. Smith.) The method makes use of the strong negative correlation between the concentrations of nitrate, NO_3, and dissolved oxygen, O_2, which exists in waters near the shelf break at depths from 50 to 300 m. This inverse relationship is evident in data presented by Fournier et al. (1977) and is quantified in Fig. 2a


a
NITRATE CONCENTRATION (µg-at·L⁻¹)
DISSOLVED OXYGEN CONCENTRATION (mL·L⁻¹)
N=-5·05 O2+33·91
b
DISSOLVED OXYGEN CONCENTRATION (mL·L⁻¹)
SALINITY (‰)
DEC. 1975
O2=-390 S+19·31
JULY 1976
O2=-538 S+23·74
OCTOBER 1976.
40–50 m avg.
50–60 m avg.

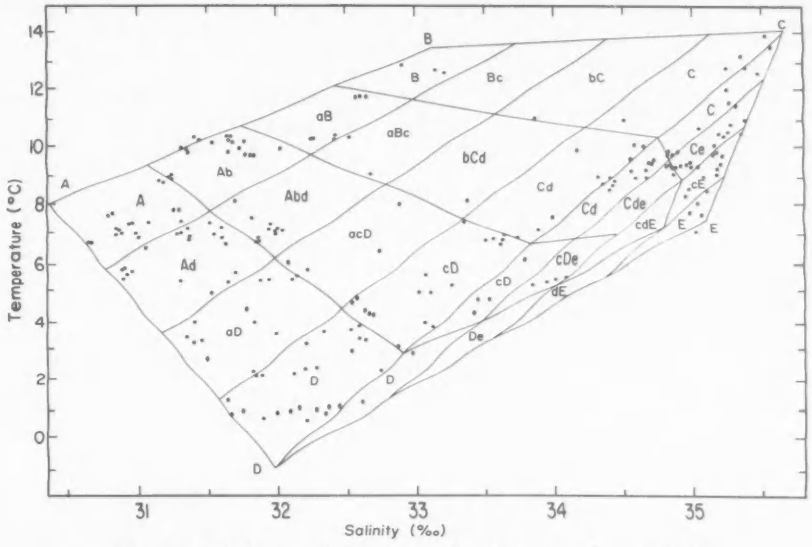


FIG. 3. *T–S* diagram of all stations with grid for water-type analysis.

for samples taken in the neighborhood of the mooring by P. C. Smith (unpublished data). Then, to relate nitrate concentrations to the current-meter data, local correlations were derived between the more densely sampled variables of oxygen, temperature, and salinity in the immediate vicinity of the mooring at the sensing depth. At 50 m, the correlation between oxygen and salinity or temperature is negative and fairly stable in three seasons (Fig. 2b). Once these relationships are established, the turbulent shoreward nitrate transport may be calculated by multiplying the corresponding salt flux, for instance, by the product of the NO_3 vs. O_2 and O_2 vs. S regressions. Since temperature is highly correlated with salinity in this region, a similar result is obtained when the former variable is used. Notice that the signs of the multiplicative factors at 50 m are such that a shoreward flux of salt or heat produces a nitrate transport in the same direction.

Results

Hydrographic Sections

The description of the water-mass distribution over the Scotian Shelf was made with a method proposed by Miller (1950) and first applied to the Scotian Shelf by McLellan (1954). In this method source waters are first identified by a small area on the temperature–salinity diagram. Then assuming that mixing is rapid compared with local and seasonal changes, the positions of points relative to the sources on the *T–S* diagram are interpreted as the results of mixing among various water masses.

Superimposed on the *T–S* diagram (Fig. 3) is a grid used in the analysis in which the corners are identified by the following water sources:

A — surface water flowing from the Gulf of St. Lawrence through the western side of Cabot Strait;

B — surface water over the slope;

C — water with maximum temperature and salinity characteristics of slope water at intermediate depths (100–150 m);

D — subsurface water (100–150 m) in the Cabot Strait;

E — deeper slope water (200–300 m).

These water classifications, although consistent with those of McLellan (1954), are appropriate for this particular cruise, and the location of points A and B are especially subject to seasonal variations. The grid delineates regions on the *T–S* diagram whose water type is attributed to mixing in various proportions of the five water types, assuming that there is no mixing of E with A or B. These regions are labeled to indicate the proportion of the constituent water type, e.g. block A is water with >75% of type A water and block Ab is water with 50–75% type A and 25–50% type B.

FIG. 2. a, Correlation between nitrate and dissolved oxygen concentrations for depths >50 m in the vicinity of the shelf-break mooring (63°30′W from 42° to 43°N) in July 1976. The correlation coefficient for 43 samples is −0.89 and the slope of the least square regression is given by $\partial NO_3/\partial O_2 = -5.05\ \mu g\text{-at}\ L^{-1} \cdot mL^{-1} \cdot L$. b, Dissolved oxygen vs. salinity at 50 m near the shelf-break mooring. Trends in discrete sample (December 1975 and July 1976) and digitized analogue (October 1976) data are compared for three different seasons. Dashed lines represent least squares regressions for discrete data. For digitized profile data, each symbol represents average O_2 value over 0.5‰ salinity range. Numbers denote samples in that range.

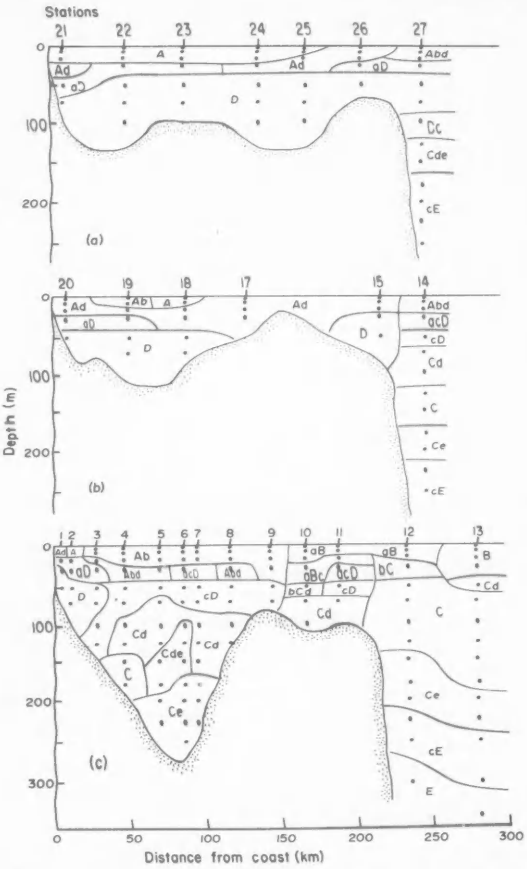


FIG. 4. Sections normal to coast showing water-type distribution: a, Banquereau; b, Sable Island Bank; c, Halifax Section. Each water sample used in the analysis is indicated by a dot and water-mass delineations are schematic.

The interpretation of the interior region is more ambiguous but the dominant water type is indicated by a capital letter, e.g. Abd. From the distribution of the points throughout this grid it is clear that most of the water on the shelf arises from mixtures of A and B, A and D, C and D, and C and E.

With water types defined in this way we now can infer the mixing and advection of the water over the Scotian Shelf from examination of their distribution (Fig. 4). The eastern section (Fig. 4a) over the Banquereau shows St. Lawrence water spread across the whole shelf and over the slope. The shelf near Sable Island (Fig. 4b) is still dominated by St. Lawrence water as A and D water become mixed. Not until the western section (Fig. 4c) do slope waters intrude over the shelf, and pure St. Lawrence water is confined to a narrow band along the coast. The E water found in Emerald Basin is probably the result of intrusion via the Scotian Gulf (see Fig. 6).

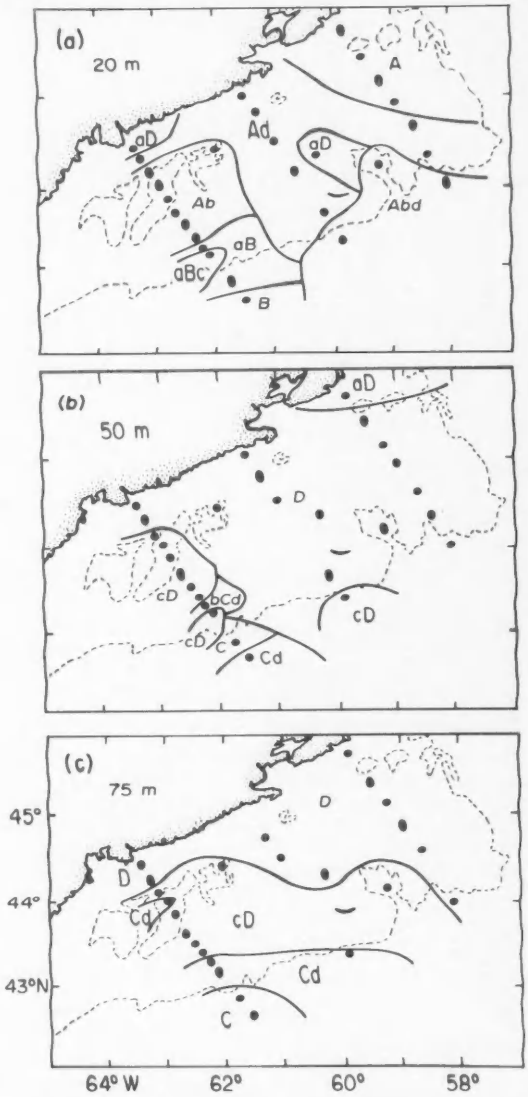


FIG. 5. Water-type distribution over shelf: a, at the 20-m depth; b, at the 50-m depth; c, at the 75-m depth. Dots indicate station location.

The horizontal distribution of the water types (Fig. 5) shows that St. Lawrence water spreads across the eastern end of the shelf, mixes vertically, and narrows to a coastal band farther west; intrusions of slope water occur west of Sable Island. Although the station spacing is not sufficient to resolve structure arising from the complex topography of the shelf, there is an indication of a local intrusion of slope water in the Gully just east of Sable Island. The main source of slope water, however, is via the Scotian Gulf and the intrusion extends

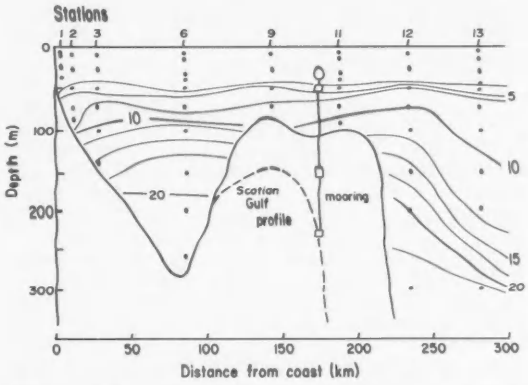


FIG. 6. Nitrate (in units of μg-at $N \cdot L^{-1}$) section through *Emerald Basin. Superimposed is the Scotian Gulf and mooring location.*

TABLE 1. Fluxes of heat, salt, and nitrates for subinertial frequencies at the shelf break.[a]

Depth (m)	Heat flux $\overline{V'T'}$ ($\times 10^2$ m·°C·s^{-1})	Salt flux $\overline{V'S'}$ ($\times 10^2$ m·‰·s^{-1})	Nitrate flux $\overline{V'N'}$ (μg-at N·m^{-2}·s^{-1})
50	4.8	1.7	38.3
150	0.5	0.3	−10.7
230	−1.1	−0.1	0.0

[a]The gradients of nitrate concentration with temperature and salinity, used to derive nitrate fluxes (P. C. Smith unpublished) from those of heat and salt, are given by

$(\partial N'/\partial T')$ (50, 150, 230 m) = (0.64, −2.26, 0.0) $\times 10^3$ μg-at N·m^{-3}·°C^{-1}

and

$(\partial N'/\partial S')$ (50, 150, 230 m) = (2.72, −3.21, 0.0) $\times 10^3$ μg-at N·m^{-3}·‰$^{-1}$.

Also, in the flux notation, overbar indicates the record mean for that quantity and prime (') indicates a deviation from the mean (e.g. $V' = V - \bar{V}$).

eastward to the far end of Emerald Basin.

The distributions of various nutrients show a similar *pattern of slope water enrichment on the shelf. The* western section of NO_3 (passing through Emerald *Basin) is shown in Fig. 6. Nitrate levels in the euphotic* zone are negligible, resulting in a very sharp gradient *at 50–60 m deep. Since nitrate levels in the St. Lawrence* water entering the eastern end of the shelf range be*tween 7 and 9 μg-at N·L^{-1}, the high nutrient levels in* Emerald Basin must be the result of fluxes through Scotian Gulf.

FLUX MEASUREMENTS

Strong shoreward subtidal heat and salt transports are *concentrated in the lowest frequencies at 50 m, whereas* the fluxes at the deeper levels are barely significant (Fig. *7). Above the inertial frequency, f, the measurements* appear to be highly contaminated by internal waves, *particularly at the M_2 tidal frequency. A distinct baro*clinic component was found in the kinetic energy dis*tribution at M_2 (P. C. Smith unpublished data) and the* reversal with depth of the sign of the cospectral esti*mates is consistent with a simple low-mode internal*

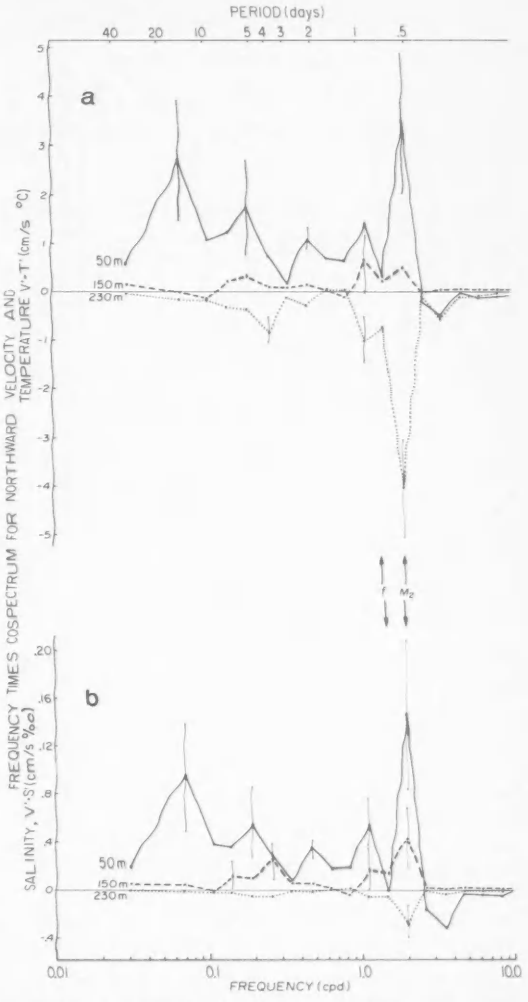


FIG. 7. Shoreward eddy fluxes at shelf-break mooring, *December 1975 to April 1976: a, Cospectrum of northward* velocity and temperature, $V'T'$; b, cospectrum of northward *velocity and salinity, $V'S'$.*

tide. For this reason, the covariance at frequencies greater than f will be excluded from the flux calcula*tions, representing a reduction of <20% at the 50-m* level.

The 50-m fluxes are shoreward and exceed those at the other depths by at least a factor of 4 (Table 1). At *150 m, the transport is weakly onshore and at the* bottom it is directed offshore. In addition, the computa*tions for the nitrate fluxes based on the local regressions* between nitrate and S(or T) properties are tabulated *for each depth. These estimates represent the average* between the values obtained by using the heat and salt *fluxes that agreed to within 40% of the mean. As noted*

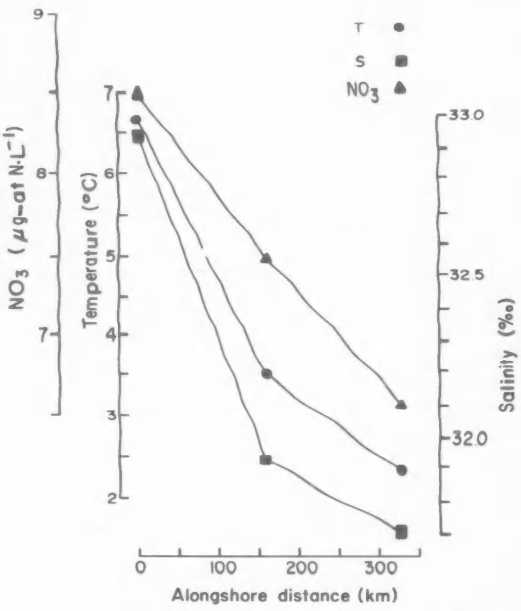


FIG. 8. Alongshore variation of temperature, salinity, and nitrate averaged over shelf.

earlier, the strong shoreward heat and salt transports at 50 m are transformed into shoreward nutrient fluxes. At 150 m, on the other hand, there is a weak offshore flow of nutrients because the O_2 vs. S(or T) regression is positive at that level. At the bottom the uniformity of O_2 values near the oxygen minimum results in negligible fluxes.

A Box Model For Cross-Shelf Mixing

To assess the compatibility of the section data with direct flux measurements we calculated alongshore gradients of temperature, salinity, and nutrients. However, the complex topography and widely spaced data make possible only rough estimates of the sectional averages for these quantities (Fig. 8). The average temperature and salinity are calculated by integrating the analog traces from the surface to the shelf floor (or to 115 m deep over Emerald Basin) for all stations from the coast to the shelf break. Since the euphotic zone is virtually depleted of NO_3, the nitrate average is calculated over depths below 50 m. The need to interpolate between stations and, for NO_3, between discrete bottle samples makes it difficult to estimate the uncertainty in these values. The overall gradient from east to west is certainly accurate to within 50%. The increased T and S gradient at the western end is evidence of greater shelf-slope water exchange via the Scotian Gulf.

Atmospheric influences such as precipitation and evaporation have little influence on the longshore gradient. Heating by solar radiation, if mixed to 100 m deep, will raise the water temperature by 0.05°C/d. During the 4 d required to complete the hydrographic sections this would produce an alongshore gradient of only 0.2°C. Since all of the water on the shelf and in the Gulf of St. Lawrence is being heated at nearly the same rate, we do not expect solar radiation to produce a significant alongshore temperature gradient. The longshore gradients of T, S, and NO_3 are all consistent with a mixing of roughly 40% slope water (type C) with the Banquereau shelf water; hence we conclude that they are primarily the result of cross-shelf mixing.

The shoreward fluxes of T, S, and NO_3 at the shelf break required to support the observed gradients on the shelf vary inversely with the mean volumetric transport alongshore. This quantity has been recently estimated on a monthly basis using geostrophic calculations performed on historical hydrographic data (K. F. Drinkwater and W. H. Sutcliffe, Bedford Institute of Oceanography, Dartmouth, N.S., personal communication). They find that the net southwesterly transport over the shelf is confined primarily to the upper 100 m and reaches a maximum of 0.56×10^6 m^3/s in January and a minimum of 0.08×10^6 m^3/s in August with an annual mean of 0.33×10^6 m^3/s. Using Fournier et al.'s (1977) figure for the average width of the shelf (188 km), this annual transport gives a mean flow of 1.5 km/d longshore in this layer. Now if the 50-m values from Table 1 are taken to represent shoreward eddy heat and salt fluxes in the top 100 m at the shelf break, then the longshore gradients in the shelf-wide average temperature and salinity may be computed (Table 2). The agreement between the computed and measured gradients (Fig. 8) is well within the uncertainty resulting from the variation in volumetric transport alone.

TABLE 2. Comparison between observed longshore gradients and productivity requirements with measured shoreward fluxes at the shelf break.

	Observed gradient + biological requirement[a] (/100 km)	Gradient supported[a] by measured flux (/100 km)
Temp	1.0°C	1.5°C
Salinity	0.4‰	0.5‰
Nitrate	0.3+0.5=0.8 μg-at N·L^{-1}	0.6 μg-at N·L^{-1}

[a]The basis for these comparisons is the simple equation $\Delta y \cdot \bar{U} \cdot (\partial\bar{\phi}/\partial x) = -\overline{V'\phi'}$, where x and y are measured along and across the shelf, respectively, Δy is the shelf width (188 km), and $\phi = T$, S, or NO_3. The mean longshore gradient is $\partial\bar{\phi}/\partial x$ and $\overline{V'\phi'}$ is the shoreward eddy transport at the shelf break. In this coordinate system the average flow $\bar{U}$ along the shelf in the top 100 m is given by $\bar{U} = Q/A = -1.5$ km/d, where $A = \Delta y \times 100\text{ m} = 1.88 \times 10^7$ m^2 and $Q = 0.33 \times 10^6$ m^3/s is the annual mean transport (K. F. Drinkwater and W. H. Sutcliffe personal communication). This value of $\bar{U}$ has also been used to convert Fournier et al.'s (1977) uptake rate to an equivalent longshore nitrate gradient.

The shoreward flux of nitrate at the shelf break must support not only the observed gradient in nitrate concentration, but also the biological requirements on the shelf. According to Fournier et al. (1977), the spring and summer nitrogen input requirement for a section 50 m deep, 188 km wide, and 1 cm thick is 15.5 μg-at $N \cdot s^{-1}$. An equivalent flux onto the shelf would produce a longshore gradient of 0.5 μg-at $N \cdot L^{-1} \cdot 100\ km^{-1}$. Combining this with the observed gradient of 0.3 μg-at $N \cdot L^{-1} \cdot 100\ km^{-1}$ means that we require a nitrate flux sufficient to produce a gradient of 0.8 μg-at $N \cdot L^{-1} \cdot 100\ km^{-1}$. Using the 50-m nitrate flux from Table 1, but applying it only over the lower half of the 100-m water column (the euphotic zone is devoid of nutrients), yields a measured nitrate gradient of 0.6 μg-at $N \cdot L^{-1} \cdot 100\ km^{-1}$. Alternatively, an estimate more appropriate for the Scotian Gulf may be derived if the average between the 50- and 150-m fluxes is applied to that 100-m layer. In this case the result (0.4 μg-at $N \cdot L^{-1} \cdot 100\ km^{-1}$) is not changed substantially.

Thus, we see that the measured fluxes in the top 100 m of water at the edge of the Scotian Shelf are consistent with the observed longshore gradients of T, S, and NO_3, and the biological uptake over the shelf. Although the longshore gradient is not exactly constant and there is evidence of enhanced cross-shelf exchange through the Gully and the Scotian Gulf, our data can only justify a simple box model as a first approximation. The agreement is remarkable, since it appears to be far better than the uncertainty in the individual quantities used in the calculation. It is noteworthy that the flux onto the shelf required to produce the longshore gradient in nitrate concentration and the nitrate uptake in the euphotic zone are comparable in magnitude. The importance of cross-shelf mixing to the water-mass characteristics and biological productivity of the Scotian Shelf is unmistakable.

Acknowledgments

We are indebted to a large number of students in the Department of Oceanography who contributed their time and effort to the success of the Dalhousie program. The technical support provided by Ms M. Van Det of Dalhousie and Mr T. Foote of Bedford Institute was invaluable. We also benefited from conversations with Drs A. Bowen, G. A. Riley, B. D. Petrie, and Mr K. F. Drinkwater. Finally, our thanks go to the captain, officers, and crew of the C.S.S. Dawson.

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Isotopic Composition of Dissolved Inorganic Carbon in the Great Lakes

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WEILER, R. R., AND J. O. NRIAGU. 1978. Isotopic composition of dissolved inorganic carbon in the Great Lakes. J. Fish. Res. Board Can. 35: 422–430.

Values for the $\delta^{13}C$ of the dissolved total inorganic carbon in the Great Lakes are presented. The surface values are about two parts per thousand more negative than the values to be expected assuming equilibrium with the atmospheric CO_2 reservoir. In the hypolimnion of Lake Erie, the values become more negative as the summer progresses due to the increasing amounts of CO_2 from decaying organic matter. Although Lakes Erie and Ontario receive considerably larger amounts of organic carbon as domestic and industrial sewage effluents than the upper Great Lakes, their higher inorganic carbon concentrations evidently mask any isotopic effects from the decay of the organic pollutants. Models to explain the variation in the $\delta^{13}C$ in the hypolimnion and epilimnion of a lake are presented. The agreement between predicted and observed $\delta^{13}C$ trends for the hypolimnion model is reasonable, suggesting that the flux rates assumed in the model are reasonable for the processes occurring in the lakes.

Key words: carbon isotopes, Great Lakes, inorganic carbon, models

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Les auteurs présentent les valeurs du $\delta^{13}C$ du carbone inorganique total dissous dans les Grands lacs. Les valeurs de surface sont d'environ deux parties par mille plus négatives que les valeurs auxquelles on pourrait s'attendre en supposant un équilibre avec le réservoir de CO_2 atmosphérique. Dans l'hypolimnion du lac Erié, les valeurs deviennent plus négatives à mesure que l'été progresse, par suite des quantités croissantes de CO_2 provenant de la matière organique en décomposition. Bien que les lacs Erié et Ontario reçoivent des quantités beaucoup plus grandes de carbone organique sous forme d'égouts domestiques et industriels que les Grands lacs supérieurs, leur teneur plus élevée en carbone inorganique masque évidemment tout effet isotopique causé par la décomposition des polluants organiques. On présente des modèles expliquant la variation du $\delta^{13}C$ dans l'hypolimnion et l'épilimnion d'un lac. Il y a assez bon accord entre les tendances prédites et observées du $\delta^{13}C$ dans le modèle de l'hypolimnion, ce qui donne à croire que les vitesses d'écoulement que suppose le modèle s'accordent assez bien avec les processus qui ont lieu dans le lac.

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WATER samples were collected from the Great Lakes between 1971 and 1974 and the isotopic ratios of ^{13}C to ^{12}C[1] in the dissolved inorganic carbon were determined. The intent was to ascertain whether the ratios changed significantly from the oligotrophic Lake Superior to the culturally eutrophic Lakes Erie and Ontario. A further aim was to study both the seasonal isotopic variability in Lakes Erie and Ontario and the effect of the development of the thermocline on the carbon isotope composition in Lake Erie. The results are discussed in terms of a deterministic model that considers the cycling of carbon isotopes among the major carbon pools in the limnetic environment.

Variations in carbon isotope composition of calcareous shells have been applied with varying degrees of success in paleoenvironmental work (Keith et al. 1964; Fritz and Poplawski 1974; Fritz et al. 1975). These studies assume that the ^{13}C content of the shells is controlled primarily by the dissolved carbon pools in freshwater environments (see Oana and Deevey 1960). The present study is also of interest in terms of paleolimnological applicability of carbon isotopic data.

[1]The ratios are usually given in the del notation: $\delta^{13}C\ (‰) = \{(R\ \text{sample}/R\ \text{standard}) - 1\}\ 1000$, where $R = {}^{13}C/{}^{12}C$.

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Methods

The sampling locations in the Great Lakes are shown in Fig. 1. The results are presented in Table 1.

In 1971 the samples were stored after collection in polyethylene bottles refrigerated at 5°C until processed

FIG. 1. Sample locations in the Great Lakes.

TABLE 1. Measured and calculated $\delta^{13}C$ values for the dissolved inorganic carbon in the Laurentian Great Lakes

Lake	Date	Measured $\delta^{13}C$ (‰) $\bar{x}$	SE	n	Equilibrium[a] $\delta^{13}C$ (‰)		C_T (mmol·dm^{-3})[b] $\bar{x}$	SE	pH $\bar{x}$	SE	Temp (°C) $\bar{x}$	SE
Erie	June 22–23	0.26E[d]	0.18	3								
Central[c]		−0.57H	0.31	3			2.18	0.02				
Basin	July 28–30	−0.76E	0.61	3								
(1971)		−1.43H	1.33	3			2.30	0.03				
	Aug. 31–Sept. 1	0.32E	0.21	3								
		−3.62H	0.13	3			2.31	0.03				
	Oct. 28–30	−0.50E	0.13	3								
		−0.76H	0.02	3			2.28	0.02				
Whole	Aug. 16–21	0.15E	0.13	12	1.03		1.82	0.03	8.74	0.04	22.3	0.2
lake	combined	−0.73H	0.92	12			1.84	0.04				
		−0.08E	0.12	24								
		−1.07H	0.26	24								
Ontario	Jan. 9	−0.43	0.14	5	2.48	1.85	0.01	8.29	—	4.8	—	
(1973)	Apr. 7	−0.59	0.16	8	2.65	1.86	<0.01	8.13	0.02	2.3	0.2	
Bay of Quinte	Aug. 30	−6.28	0.38	5	0.64	1.90	0.02	8.74	0.05	26.0	—	
(1973)	Sept. 6–7	−3.77	0.37	7	0.77	1.85	0.02	8.82	0.03	23.9	0.9	
	combined	−4.81	0.45	12								
Superior	June 2–5	0.30	0.23	7	2.34	0.95	0.02	7.90	0.03	4.0	—	
(1974)												
Huron[e]	June 24–28	−0.32	0.23	7	1.80	1.42	0.54	8.04	0.05	6.4	1.2	
(1974)												
Georgian Bay	June 18	0.12	0.22	2	1.18	1.68	0.03	7.70	—	5.0	—	
(1974)												

[a]Calculated values at the in situ alkalinity, temperature, and pH assuming that the waters are in isotopic equilibrium with the atmospheric pool of CO_2 that has a $\delta^{13}C$ value of −7 ‰.

[b]Total dissolved inorganic carbon calculated from alkalinity and pH at the in situ temperature or measured directly by gas chromatography.

[c]The central basin stations are E-11, G-16, and L-19; the whole lake stations are 1–50.

[d]E-epilimnion; H-hypolimnion, samples taken within 0.5–1 m from the bottom.

[e]Two values of −2.52 and −3.06 ‰ for station 6 are not included, as they probably represent outflow of Saginaw Bay water.

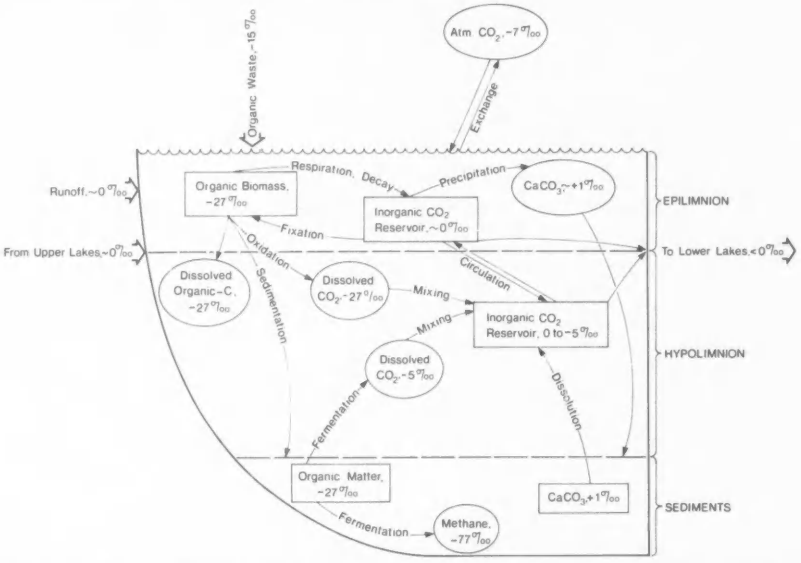

FIG. 2. Pathways and the isotopic effects of the cycling of carbon between the major pools in a stratified lake.

within a week of collection. After pressure filtering the sample in a nitrogen atmosphere, the total inorganic carbon was precipitated using the strontium carbonate method of Friedman (1970). In subsequent years, the samples were collected in glass bottles sealed with greased stoppers and poisoned with $HgCl_2$. The samples were refrigerated for up to a month before being processed. The total inorganic carbon was stripped using a vacuum system similar to that of Kroopnick (1974). Dr H. P. Schwarcz of McMaster University, Hamilton, Ontario, determined the isotope ratios of the Lake Erie and Lake Ontario samples. Ratios for the other samples were measured on a VG Micromass 602 mass spectrometer of our laboratories. All $\delta^{13}C$ values are corrected for O^{17} contribution to peak 45 and quoted relative to the PDB standard.

To find out if the precipitation method produced results that were systematically different from the stripping method, a sample of tap water was treated both ways. There was no clear evidence for systematic differences. The same experiment showed that the samples precipitated by $SrCO_3$, had a standard deviation of the mean (SE) of 0.2–0.6‰ for replicates and around 0.2‰ for duplicates. For the samples where the CO_2 was extracted directly, SE for replicate samples was about 0.01‰ and for duplicates about 0.1‰.

There is an indication, based on only one set of replicate measurements, that water samples kept in the dark and refrigerated but stored in plastic bottles become heavier by about 0.3‰ whereas samples kept under the same conditions but in glass bottles do not show any change over a period of 8 days. If this is generally true, then the 1971 samples should be corrected by subtracting 0.3‰ from the values given in Table 1.

The measured $\delta^{13}C$ values given in Table 1 are unweighted averages of all samples collected in a lake or a segment of it. With the exception of Lake Erie, there were no apparent systematic differences with depth. The Lake Erie samples were collected either within 1 m of the surface (epilimnion) or in the hypolimnion within 0.5–1 m of the bottom.

The concentrations of the dissolved carbon species were calculated from either the alkalinity and pH or total carbon and pH at the in situ temperature, using Lyman's apparent dissociation constants at 0 chlorinity (Riley and Skirrow 1965). Comparison of the total carbon (C_T) values measured directly by gas chromatography and those calculated from alkalinity and pH show that they differ by less than 0.1 mmol·dm^{-3} on the Lake Erie cruises. Weiler (1975) also showed that there are no systematic differences between the partial pressures of dissolved CO_2 measured directly and those calculated from pH and alkalinity using Lyman's constants.

Results

The pathways in the cycling of carbon among the major carbon pools in any of the Great Lakes are shown schematically in Fig. 2, which is based on Fig. 1 in Oana and Deevey (1960). If the lake has reached isotopic equilibrium with the atmosphere, the distribution of ^{13}C among the various carbon species and the $\delta^{13}C$ of the total dissolved inorganic carbon can be calculated from the equations in Deines et al. (1974). Since the value for atmospheric CO_2 was not determined, the value used in the calculation was −7‰ (Keeling 1958, 1961), although this value is strictly correct only for air with a volume percent of CO_2 around 0.0315. As the volume percent increases, the $\delta^{13}C$ of atmospheric CO_2 becomes more negative. Since the volume percent around the lakes can be expected to be higher because of atmospheric inputs

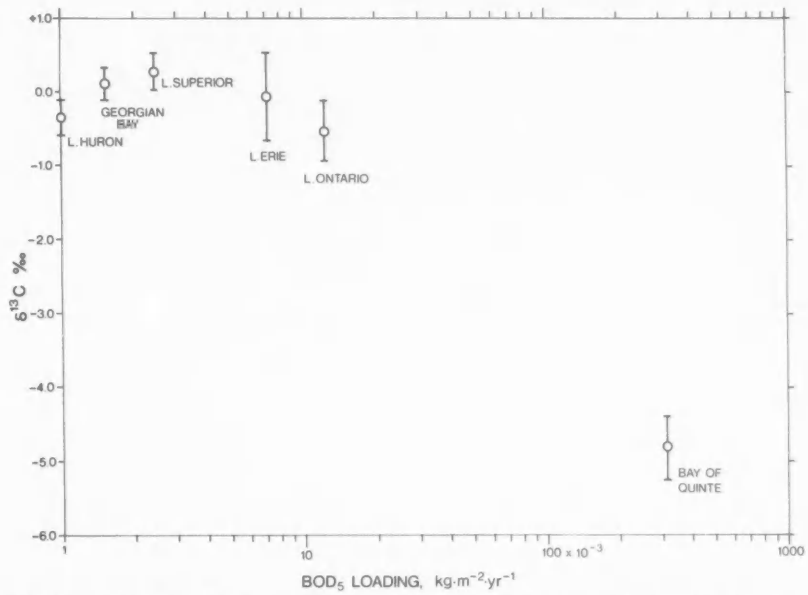

FIG. 3. Relationships between average $\delta^{13}C$ in the Great Lakes and the BOD_5 loadings. The bars represent 1 SE.

(Weiler 1974; Allee et al. 1970), the $\delta^{13}C$ is probably less than −7‰. In the equations used by Deines et al. (1974), the $\delta^{13}C$ of the dissolved inorganic carbon varies directly with the $\delta^{13}C$ of the atmospheric CO_2. Calculations have shown that for the Great Lakes, a decrease of 1‰ in atmospheric CO_2 will lead to a decrease of 1‰ in the dissolved carbon. Therefore, the equilibrium $\delta^{13}C$ values in Table 1 are probably the maximum ones. Further, although the atmospheric $\delta^{13}C$ over land can vary considerably, short-term fluctuations in dissolved carbon are smoothed out by buffering capacity of the lakes.

The measured $\delta^{13}C$ values are, with a few exceptions, lighter than the calculated equilibrium values. This should be expected since the $\delta^{13}C$ contents of the dissolved carbon pool are also influenced by biological activities (for example see Sackett and Moore 1966; Mook 1970). Respiration of plankton and the decay of detritus will produce CO_2 with δ^{13} values ranging from −20 to −80‰. Although detrital matter settles through the thermocline, the light dissolved carbon will be brought to the surface when the lakes mix from top to bottom in both spring and fall. Photosynthesis, on the other hand, removes CO_2 depleted in $\delta^{13}C$ (Sackett and Moore 1966).

Another factor influencing the value is the considerable amount of organic carbon received by Lakes Erie and Ontario as domestic and industrial sewage effluents (IJC 1969). The biodegradation of the large load of pollutant organics should result in the inorganic carbon reservoirs of these lakes being lighter than those of Lake Superior. For example, anthropogenic organic materials with values in the range of −15 to −25‰ have been implicated as being responsible for the depletion of ^{13}C in the water of Lake Erie (Fritz et al. 1975), the lower New York Bay (Sackett and Moore 1966), and in the Bedford Basin (Tan and Walton 1975). To test this hypothesis the 5-d biological oxygen demand (BOD_5) was used as an approximation for the man-made organic loading. The information was obtained from the following sources: Lake Erie, Lake Ontario, and Bay of Quinte — IJC Report (1969); Lake Huron, Lake Superior, and Georgian Bay — IJC Report (1976). The average $\delta^{13}C$ value (Table 1), excepting the Lake Erie hypolimnion values, were plotted as a function of the BOD_5 loading (Fig. 3). With the exception of the Bay of Quinte, there is no noticeable effect caused by increased BOD_5 loading.

Because of the calcareous nature of their drainage basins, Lakes Erie and Ontario also have considerably higher dissolved inorganic carbon content than the upper Lakes, as well as higher calcium and magnesium concentrations (Weiler and Chawla 1969). Part of the inorganic carbon is also derived from the dissolution of detrital calcite and dolomite, the $\delta^{13}C$ of which is close to 0. These factors apparently mask any isotopic effects caused by the pollution of the lower Great Lakes with organic carbon. Figure 4 shows the variation of $\delta^{13}C$ with total dissolved inorganic carbon. An exception to the suggested masking effect is the Bay of Quinte, a long, narrow water body that, in addition to receiving high BOD_5 loadings, also receives nitrogen and phos-

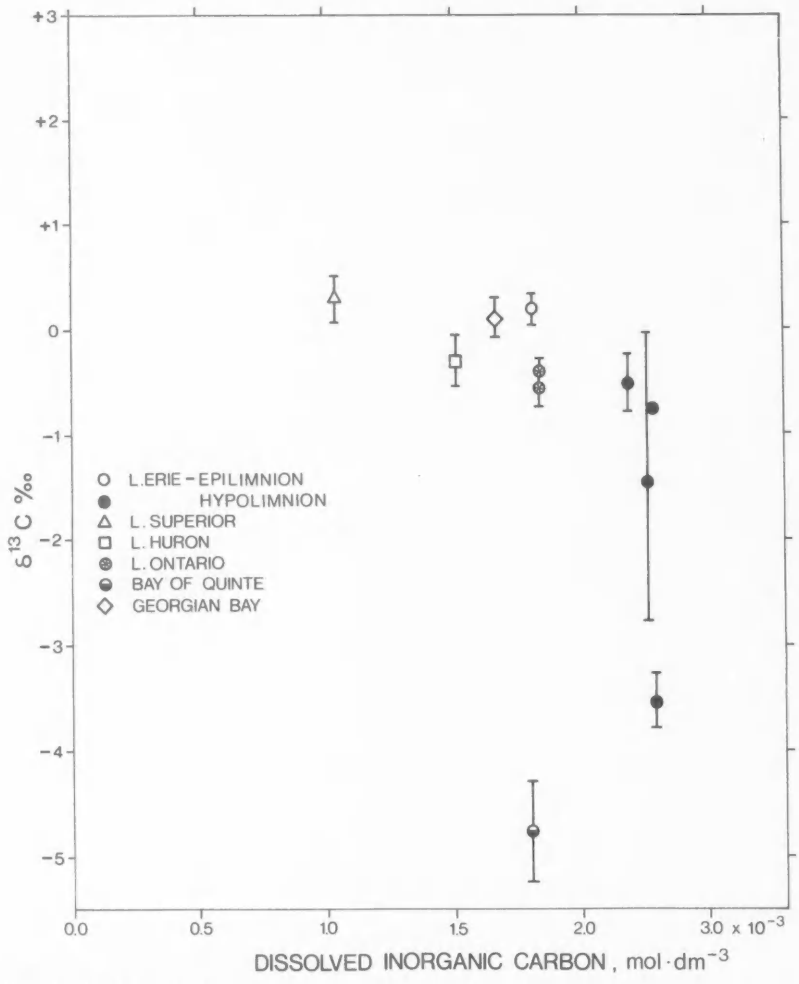

FIG. 4. Relationship between average $\delta^{13}C$ on the Great Lakes and the concentration of total dissolved inorganic carbon. The bars represent 1 SE.

phorous inputs per unit area exceeding those of Lake Erie (Johnson and Owen 1971). Another example may be station 6 in Lake Huron, where $\delta^{13}C$ values of -2.5 to -3.1 suggest the presence of water from highly polluted Saginaw Bay.

The δ values of the hypolimnion of Lake Erie (in Table 1 the samples designated with H) become more negative as the year progresses, and then equal those of the surface samples again in the fall (Fig. 5 and Table 1; see also Fig. 6 for individual stations in the central basins of Lake Erie). The epilimnion value, however, remains constant over the seasons, as far as can be deduced from the scattered data available. In qualitative terms, this can be explained as follows. The thermocline, which severely restricts exchange of water between the epilimnion and hypolimnion of the lake, forms in early June and descends to its maximum depth by the end of the month. The concentration of inorganic carbon increases since the CO_2 produced by decaying organic matter both in the hypolimnion and in the bottom sediments cannot escape to the atmosphere. Consequently, the δ values become more negative. By October, the autumn storms have destroyed the thermocline and mixed the hypolimnion waters with those of the epilimnion.

The other factors that can affect the isotopic composition of the dissolved inorganic carbon are inflow from rivers, rain falling directly on the lake surface, pre-

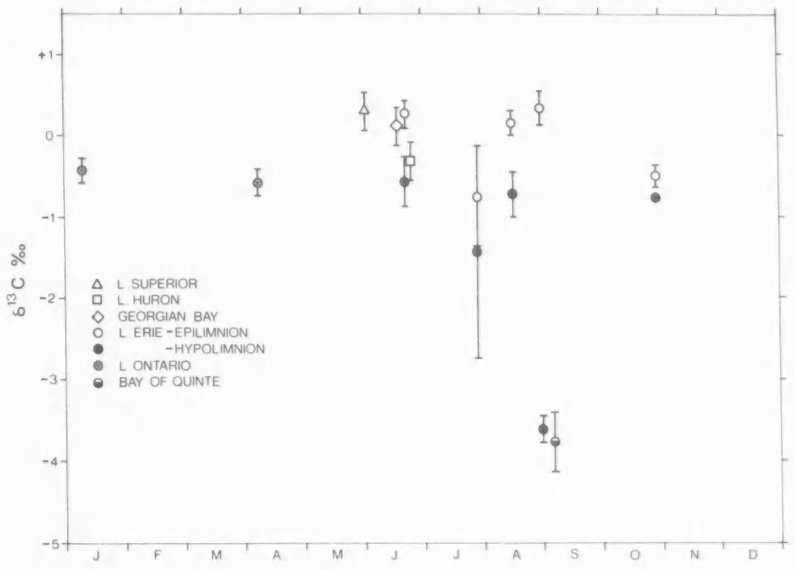

FIG. 5. Seasonal variation of $\delta^{13}C$ on the Great Lakes. The bars represent 1 SE.

cipitation of carbonates in the epilimnion and their redissolution in the hypolimnion, and the residence time of water in the lakes.

Since rainwater contains very little dissolved inorganic carbon, its contribution to the isotopic composition of the lakes is minimal. There are no $\delta^{13}C$ measurements for Great Lakes tributaries, but rivers are generally lighter than lakes (for example see Mook 1970). Nevertheless, the effect of the tributaries must be small, as the $\delta^{13}C$ value in Lake Superior, which receives 46% of its water input from tributaries, does not differ markedly from the $\delta^{13}C$ in Lake Erie, which receives only 10% from tributaries (IJC 1969, 1976).

In the summer, the surface water of Lake Huron, Lake Erie, and Lake Ontario is supersaturated with respect to calcite, aragonite, and dolomite (Weiler and Chawla 1969). If extensive precipitation and redissolution were to occur, an enrichment of calcium below the thermocline should be expected. Unpublished data collected by CCIW do not show any significant differences between the epilimnion and hypolimnion (see also Weiler and Chawla 1968, for data from the central basin of Lake Erie). Therefore, this process probably has only a minimal effect on the isotopic composition.

In conclusion, therefore, Lake Superior approaches equilibrium with the atmospheric CO_2 through gas exchange and subsequent fractionation (Deuser and Degens 1967), because of the long residence time of the lake, which is 113 yr (IJC 1976). Mook (1970) has suggested that 2–6 mo are needed for equilibrium to be reached in a lake.

The major sources of water for the downstream lakes are the upstream lakes and because of the large buffering capacities, relatively long flushing times (Lake Huron, 17 years; Georgian Bay, 7 years; Lake Erie, 2.6; Lake Ontario, 7.9; IJC 1969, 1976), and the complete mixing of the lake from the surface to the bottom in late fall and spring, the other processes outlined above do not appear to affect the average isotopic composition materially. Exceptions are the Bay of Quinte and the hypolimnion of the central basin of Lake Erie.

Model for $\delta^{13}C$ Changes in Hypolimnion of Lake Erie

It is possible to formulate a simple model to explain the ^{13}C variations in the hypolimnion of the lake. Conceptually this model is the equivalent of the closed systems of Deines et al. (1974). It is assumed in this model that the hypolimnion is isolated from the atmosphere and that the only process changing the amount of dissolved inorganic carbon and isotopic composition of inorganic carbon pool is the decay of organic material in the hypolimnion and the bottom muds. Other processes, like fermentation of organic material or advection, are not considered.

The two equations that can be used to describe these processes in the hypolimnion are:

$$(1) \qquad (C_T)_f = (C_T)_i + (\Delta C_T)_d$$

$$(2) \qquad (\delta^{13}C)_f = \{(C_T)_i(\delta^{13}C)_i + (\Delta C_T)_d(\delta^{13}C)_{org}\}/(C_T)_f$$

where C_T refers to the total dissolved inorganic carbon, $(\Delta C_T)_d$ is the change due to production of CO_2 from decay and respiration, the subscripts i and f signify

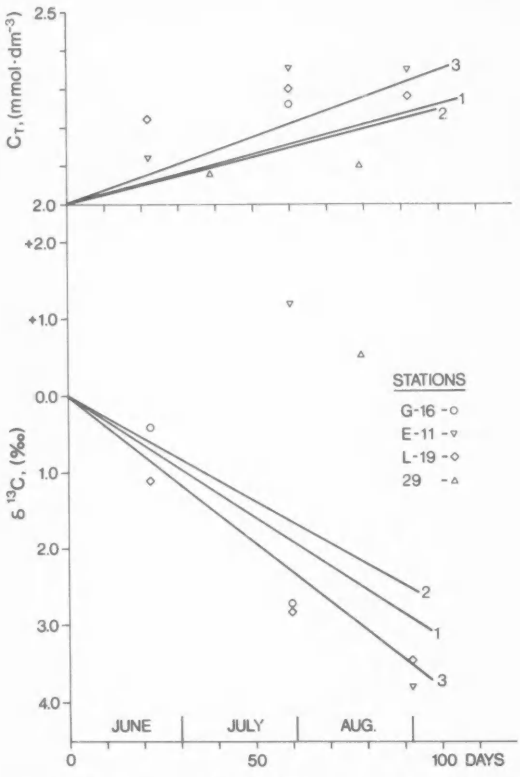

FIG. 6. Variations of dissolved inorganic carbon (C_T) and $\delta^{13}C$ in the hypolimnion of Lake Erie. Measured values and predictions based on (1) organic carbon settling rate of 0.25 g $C \cdot m^{-2} \cdot d^{-1}$, (2) oxygen depletion rate of 0.11 g $O_2 \cdot m^{-3} \cdot d^{-1}$, and (3) oxygen depletion rate of 0.15 g $O_2 \cdot m^{-3} \cdot d^{-1}$.

initial and final conditions, and org refers to the pool of organic carbon.

The calculations were done on the basis of a column of water 1 m^2 in cross section and having a length equal to the average thickness of the hypolimnion in the central basin of Lake Erie from June 1 to the end of August.

Because temperature, isotopic composition, and chemical data for the month of May and the beginning of June, 1971 are lacking, certain simplifying approximations have been made.

i) The average hypolimnion thickness from July 5 to 8 is about 5.6 m; from August 17 to 21 about 3.3. Historical data suggest that a well-defined thermocline starts to form in the latter part of May, descends quite rapidly to almost its maximum depth in the first half of June, and then deepens at a slower rate throughout the remainder of the summer. Extrapolating from the two available data points gives a hypolimnion thickness of about 8 m at the beginning of June and 3 m by the end of August. Thus, the average depth for the whole 90-d period is about 5.5 m, assuming a linear descent rate.

ii) An examination of the $\delta^{13}C$ values in Table 1 suggests that the δ value in May is not far from 0‰. During the August cruise, the C_T value for the epilimnion, which is depleted in inorganic carbon by phytoplankton growth, is about 1.95 mmol·dm^{-3}. Thus, a value of about 2.0 mmol·dm^{-3} is a reasonable concentration for the end of May. The starting concentration, $(C_T)_i$, is thus assigned the value of 132 g $C \cdot m^{-2}$.

iii) Oana and Deevey (1960) give a value of −30‰ for respiratory CO_2 from oxidation of seston. Values from −21.6 to −26.1 have been measured for zooplankton on Lake Erie and Lake Ontario (Drimmie et al. 1974). The value chosen for this model is −25‰.

$(\Delta C_T)_d$ was calculated in two ways. For the first case, an organic carbon settling rate from epilimnion to hypolimnion of 0.25 g $C \cdot m^{-2} \cdot d^{-1}$ and a 75% regeneration rate are assumed (N. M. Burns, CCIW, personal communication). Thus, the amount regenerated is 0.19 g $C \cdot m^{-2} \cdot d^{-1}$ or 16.9 g $C \cdot m^{-2}$ for the 90-d period. The final concentration is then 149 g $C \cdot m^{-2}$ or 2.26 mmole·dm^{-3}. The $(\delta^{13}C)_f$ is, from equation (2), 16.9(−25)/149 or −2.84‰.

Burns (1976) gives two volumetric depletion rates for oxygen in the hypolimnion of Lake Erie in 1970. If there is no interaction between the hypolimnia of the central and eastern basins of the lake, the rate is 0.11 g $O_2 \cdot m^{-3} \cdot d^{-1}$. With interaction, the rate increases to 0.15 g $O_2 \cdot m^{-3} \cdot d^{-1}$. These rates are equivalent to 0.61 and 0.83 g $O_2 \cdot m^{-2} \cdot d^{-1}$ for a hypolimnion 5.5 m thick.

In an earlier publication (Burns and Ross 1972), it was estimated that about 88% of the observed oxygen depletion is caused by decay processes; that is, either 0.53 or 0.73 g $O_2 \cdot m^{-2} \cdot d^{-1}$. These are equivalent to 0.017 or 0.023 mmol·$m^{-2} \cdot d^{-1}$. He also suggests that a respiratory quotient of 0.85 is reasonable. Therefore, the amount of O_2 converted to CO_2 is 0.014 or 0.019 mmol·$m^{-2} \cdot d^{-1}$, which is equivalent to 0.17 or 0.23 g $C \cdot m^{-2} \cdot d^{-1}$.

For a 90-d period, the lower value gives rise to a $(C_T)_f$ of 147 g $C \cdot m^{-2}$ or 2.23 mmol·dm^{-3} and a $(\delta^{13}C)_f$ of −2.60‰ and the higher value to a $(C_T)_f$ of 153 g C·m or 2.31 mmol·dm^{-3} and a $(\delta^{13}C)_f$ of −3.38‰

The results of these calculations together with the measured C_T and $\delta^{13}C$ for various stations in the central basin (G-16, E-11, L-19, and 29) are plotted in Fig. 6. There is a reasonable agreement between the observed and calculated trends in $\delta^{13}C$ and C_T. The carbon isotope results, therefore, lend support to the oxygen depletion rate suggested by Burns (1976) and some of the assumptions used in its derivation (Burns and Ross 1972).

Model for $\delta^{13}C$ Variations in the Epilimnion

For the epilimnion, an additional term to represent gas exchange across the lake surface has to be included.

If, as before, exchange across the thermocline is considered as negligible and horizontal advection terms are also neglected, then $(C_T)_f$ can be expressed, analogous to equation (1), as

$$(3) \qquad (C_T)_f = (C_T)_i - \Delta C_{GP} + \Delta C_R + \Delta C_{EX}$$

where ΔC_{GP} is the change in dissolved CO_2 caused by gross production, ΔC_R that caused by respiration, and ΔC_{EX} that due to exchange with the atmosphere.

Similarly (cf. equation 2), $\delta^{13}C_f$ is

$$(4) \quad (\delta^{13}C)_f = \{(C_T)_i(\delta^{13}C)_i - (\Delta C_{GP})(\delta^{13}C)_{org} + (\Delta C_R)(\delta^{13}C)_{org} + (\Delta C_{EX})(\delta^{13}C)_{EX}\}/(C_T)_f$$

The same concept was used by Schindler and Fee (1973) in discussing diurnal variations in dissolved inorganic carbon in a small lake. Gross production is defined as the sum of respiration and net production.

When algae utilize dissolved inorganic carbon, they fractionate it. Therefore, ΔC_{GP} should be multiplied by the delta value of organic matter produced. When CO_2 exchanges with the air, the $\delta^{13}C$ is taken to be that of $(CO_2)aq$.

Unfortunately, there is a lack of experimental data where all the individual terms in equations (3) and (4) — gross production, respiration, exchange, as well as the $\delta^{13}C$ value — have been measured. The model cannot, at present, be tested.

Discussion

Man has a greater impact on the lower Great Lakes than on the upper through increased inputs of nutrients and organic matter. Nevertheless, the average $\delta^{13}C$ of the dissolved inorganic carbon does not change from lake to lake, except in certain isolated segments, such as the hypolimnion during the summer in the central basin of Lake Erie or the Bay of Quinte.

Apparently the dissolved inorganic carbon of Lake Superior attains an isotopic steady state with the various inputs and outputs of carbon. The main input of water to the lower lakes is from the upper and the inputs and outputs counter balance each other so that the $\delta^{13}C$ of the inflowing waters is not modified.

The thorough mixing of the lakes from surface to bottom in the spring and early winter helps to maintain the isotopic uniformity of the lake, so that even the hypolimnion water of the central basin of Lake Erie has only a transitory effect. Only in isolated bays, such as the Bay of Quinte and possibly Saginaw Bay, is the $\delta^{13}C$ appreciably and permanently affected by man's activities and there is a significant shift in the isotopic composition of the dissolved carbon pool.

These conclusions are based on relatively few data points. The spatial and temporal variability in the water should be checked by more thorough sampling. The isotopic ratio of the air around the lakes should be measured so that the equilibrium $\delta^{13}C$ can be calculated accurately. And finally, the isotopic composition of the various carbon pools — organic and inorganic — should be measured so that a more extensive model of the isotopic composition of the Great Lakes can be formulated and checked.

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Effect of Graded Levels of Supplemental Ascorbic Acid in Practical Diets Fed to Rainbow Trout (*Salmo gairdneri*)

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HILTON, J. W., C. Y. CHO, AND S. J. SLINGER. 1978. Effect of graded levels of supplemental ascorbic acid in practical diets fed to rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 35: 431–436.

Deficiency symptoms, including anorexia, lethargy, lying prostrate at the bottom of the tank, scoliosis, and lordosis, were noted 20 wk after the start of the experiment in rainbow trout (*Salmo gairdneri*) held at 15°C and fed a diet with no supplemental ascorbic acid. The apparent requirement was 40 mg/kg of supplemental ascorbic acid; however, the amount added to trout diets should be in excess of this level due to processing and storage losses and other factors. The ascorbic acid requirement for rainbow trout appears to vary with the age and rate of growth of the trout in that the requirement is higher in young fish. Ascorbic acid was noted to function in the iron metabolism of rainbow trout. Liver and spleen iron levels were correlated with the ascorbic acid level in the diet and suggest either a redistribution of iron stores due to impaired release of iron from reticuloendothelial stores or an increase in the efficiency of intestinal iron absorption. Scorbutic trout developed a gradual but progressive anemia.

Key words: ascorbic acid, rainbow trout, vitamin requirement, iron, mineral metabolism, anemia

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Nous étudions les effets de niveaux gradués d'acide ascorbique dans un régime alimentaire pratique offert à des truites arc-en-ciel (*Salmo gairdneri*) élevées à 15°C. Nous observons des symptômes de carence, y compris anoréxie, léthargie, état de prostration sur le fond du bassin, scoliose et lordose chez des poissons nourris au régimé dépourvu d'un supplément d'acide ascorbique, 20 sem après le début de l'expérience. La quantité d'acide ascorbique supplémentaire requise semble être 40 mg/kg; cependant, on devrait ajouter au régime alimentaire des truites des quantités supérieures à cette concentration afin de compenser les pertes causées par la transformation et l'entreposage, et autres facteurs. La quantité d'acide ascorbique requise par la truite arc-en-ciel semble varier en fonction de l'âge et de la vitesse de croissance, la quantité requise étant plus grande chez les jeunes poissons. Nous constatons que l'acide ascorbique joue un rôle dans le métabolisme du fer de la truite arc-en-ciel. Les niveaux de fer dans le foie et la rate sont en corrélation avec le niveau d'acide ascorbique du régime alimentaire, ce qui suggère soit une redistribution des provisions de fer quand la mise en liberté du fer emmagasiné dans les tissus réticuloendothéliaux est entravée ou quand l'intestin absorbe le fer plus efficacement. Nous constatons qu'il se développe chez les truites scorbutiques une anémie progressive.

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ASCORBIC acid is an essential nutrient for rainbow trout (*Salmo gairdneri*) (Kitamura et al. 1965; Halver et al. 1969). The ascorbic acid requirement (NRC 1973) was based upon semipurified diet studies, but rainbow trout fed practical diets may have a different dietary requirement for the vitamin. Hilton et al. (1977) showed that processing and storage losses of supplemental ascorbic acid in practical trout diets can be very severe depending upon processing methods, storage conditions, moisture content of the diet, and level of supplementation. Furthermore, fish meals and oils used in practical diets contain, to a greater or lesser degree, certain environmental toxicants, some of which, e.g. toxaphene, increase the ascorbic acid requirements of catfish (Mayer et al. 1978). Therefore we wished to determine the minimum ascorbic acid requirements of rainbow trout grown on a practical diet.

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Materials and Methods

DIET PREPARATION

Eight diets were prepared by a formula that was the same as the control diet of Cho et al. (1976) with geometrical increases of supplemental ascorbic acid (ethyl cellulose coated ascorbic acid 97.5% dry wt, Hoffmann-LaRoche Ltd., Vandreuil, Que.) from 0, 20, to 1280 mg/kg as outlined in Table 1. Diets were processed by the type 2 laboratory processing method described in Hilton et al. (1977). Diets were prepared as required every 5–8 wk and were periodically analyzed after processing as described in Hilton et al. (1977). Each diet was fed to triplicate groups of 150 fish for 11 periods, each period lasting

TABLE 1. Mean weight gains (±SD), feed–gain ratios (±SD), and mortalities (±SD) of rainbow trout after 11, 28-d periods; the data are means of three replicates × 150 fish × 11 periods and 22 173–24 562 fish-days per treatment (initial body weight 667 ± 10 g/100 fish).

Group no.	1	2	3	4	5	6	7	8
Ascorbic acid supplement (mg/kg)	0	20	40	80	160	320	640	1280
Gain (kg/100 fish)	15.8±1.3	34.2±1.3	35.8±0.4	35.6±1.7	34.6±1.3	35.2±1.1	35.7±1.6	34.4±0.8
Feed:gain	1.89±0.06	1.53±0.09	1.48±0.01	1.49±0.07	1.47±0.02	1.49±0.02	1.50±0.02	1.52±0.01
Mortality[a]	5.1±0.9	1.7±1.0	1.6±0.7	1.6±0.7	3.1±0.6	2.0±0.5	4.3±0.5	3.1±0.3

[a]Based upon number of mortalities per 10 000 fish-days.

28 d. All groups were fed a control diet supplemented with 400 mg/kg ascorbic acid for 4 wk preceding the experiment.

SUPPLY AND MAINTENANCE OF FISH

The rainbow trout were obtained from the Ontario Ministry of Natural Resources, Normandale Hatchery. The tanks were individually aerated and the water recirculated (10–20% replacement) on a biological filter system. Water temperature was maintained at 15 ± 0.58°C, and the pH varied between 7.7 and 7.8. Dissolved oxygen and total ammonia were determined twice weekly; these ranged from 8.4 to 6.8 μg/L and 0.15 to 1.45 μg/L, respectively, throughout the test periods.

GROWTH AND BIOCHEMICAL ASSAYS

Body weights, feed–gain ratios, and total mortalities were determined after each period. After period 11 the data were subjected to analysis of variance (Steel and Torrie 1960). Fish were randomly selected at the end of each period and certain tissues and blood samples taken for biochemical analyses. Total body carcass, liver, and head kidney tissues were removed and assayed for total ascorbic acid concentration by the method of Aeschbacher and Brown (1972). Blood was collected in sodium heparinized tubes after transection of the caudal peduncle, immediately centrifuged, and the serum stored at −20°C until required for analysis. Serum iron levels were determined by the method of Caraway (1963). Hemoglobin concentrations were determined by the cyanmethemoglobin method (Unopette test 5857 Becton, Dickinson & Co. Ltd., Mississauga, Ont.) and hematocrits using heparinized microhematocrit tubes (Hesser 1960). Liver iron levels (periods 5, 6, 9) and spleen iron levels (period 9) were determined by atomic absorption spectrophotometry.

TABLE 2. Carcass total ascorbate concentrations (mean of three carcass samples, each sample one to three pooled fish with an initial carcass concentration of 120 ± 15 μg/g).

Group no.	Period			
	1	3	5	7
	(μg/g ± SD)			
1	80±14	41±2	17±2	17±6
2	81±6	44±15	20±6	17±2
3	77±11	33±18	21±6	20±8
4	72±3	29±4	24±6	19±3
5	71±8	44±16	20±6	22±9
6	72±3	42±5	23±5	34±3
7	86±6	38±7	24±4	27±5
8	92±5	53±12	30±1	39±3

TABLE 3. Liver total ascorbate concentration (mean of three pooled samples of five fish each).

Group no.	Period				
	3	5	7	9	11
	(μg/g ± SD)				
1	17±2	14±4	19±8	19±8	11±1
2	22±3	14±5	14±1	14±7	15±2
3	13±3	21±3	20±4	23±5	10±3
4	24±11	34±7	32±6	24±4	20±3
5	24±4	29±6	35±10	34±6	21±9
6	23±5	37±2	42±4	49±11	29±9
7	30±7	53±6	59±14	70±4	64±3
8	55±3	80±7	79±5	127±8	108±12

Results

Deficiency symptoms appeared in group 1 (zero supplement) during period 5. The initial major symptoms were anorexia, lethargy, and lying prostrate at the bottom of the tank. Scoliosis and lordosis, although not the initial major symptoms, predominated by the end of the experiment. Only 80–85% of the fish in group 1 exhibited overt deficiency symptoms at one time. At the end of period 11, group 1 had a significantly lower weight gain and higher feed–gain ratio (Table 1) than the other groups.

During period eight, group 2 (20 mg/kg) also exhibited deficiency symptoms similar to the initial major symptoms noted in group 1. The incidence of affected fish in the individual replicates of group 2, however, seldom exceeded 10% and at the end of period 11 no significant difference was noted in the weight gain or feed–gain ratio of this group in comparison with the remaining six groups. None of the remaining six groups exhibited deficiency symptoms, and mortality rates were essentially the same in all groups (Table 1).

Carcass ascorbic acid concentrations dropped from an initial high of 120 μg/g to 17 μg/g in group 1 at the time deficiency symptoms appeared (Table 2). Liver ascorbic

TABLE 4. Head kidney total ascorbate concentration (mean of three pooled samples of two to five fish each).

Group no.	Period 5	7	9	11
	(μg/g ± SD)			
1	24±6	25±5	27±8	22±5
2	24±4	26±7	24±1	23±7
3	29±9	26±2	26±3	12±7
4	34±3	33±4	35±3	29±14
5	24±3	32±1	36±2	34±4
6	31±2	44±10	66±3	60±5
7	36±6	56±18	88±6	100±3
8	65±8	101±15	123±10	144±24

acid concentrations (Table 3) were relatively stable in groups 1 and 2, remaining below 20 μg/g from period 4 until the end of the experiment. Groups 3–8 had higher liver ascorbic acid concentrations than groups 1 and 2 except during periods 3 and 11. The liver ascorbic acid concentrations of groups 3–8 generally increased as the experiment progressed except at the end of period 11, when liver ascorbic acid concentrations declined for practically all groups. Head kidney ascorbic acid concentrations (Table 4) were also stable in groups 1 and 2 from period 5 until the end of the experiment; groups 3–8 had higher ascorbic acid concentrations than 1 and 2, which were also noted to increase as the experiment progressed except for a decline at the end of period 11 in all groups but 7 and 8.

Hemoglobin and hematocrit levels (Table 5) of group 1 decreased steadily from period 8 on, and by the end of period 11, fish exhibiting overt deficiency symptoms in group 1 had much lower hemoglobin levels and hematocrits than the other fish in this group, which appeared unaffected. The hemoglobin–hematocrit ratios showed a marked decline in group 1 as opposed to the other groups at the end of period 11. The remainder of the groups had relatively stable hemoglobin levels, hematocrits, and hemoglobin–hematocrit ratios throughout the experiment.

TABLE 6. Serum iron concentrations (mean of four pooled samples, three to five fish per sample).

Group no.	Period 6	9	11
	(μg/100 mL ± SD)		
1	100±30	81±20	50±30
2	123±9	138±23	120±27
3	138±22	127±14	132±31
4	129±16	134±16	122±16
5	137±20	118±20	119±19
6	161±27	124±14	124±18
7	153±20	126±12	128±17
8	150±20	131±6	132±16

TABLE 7. Liver (3 pooled samples; 6 livers per sample, period 5; 4 livers per sample, periods 6 and 9) and spleen (11 pooled spleens per group, no replication possible) iron concentrations.

Group no.	Period 5	6	9	9
	(μg/g dry wt ± SD)			
				Spleen
1	125	175±5	286±17	1204
2	156	118±13	270±23	821
3	149	126±5	262±44	894
4	162	160±12	348±63	837
5	165	189±14	346±44	795
6	172	198±17	306±28	743
7	186	237±2	360±25	581
8	215	266±5	449±60	681

Serum iron levels (Table 6) were variable but group 1 had consistently lower concentrations than the remaining groups from period 6 to the end of the experiment. Liver iron levels (Table 7) showed an increase in concentration with increasing dietary ascorbic acid levels. All three periods examined showed significant regressions at $P \leq 0.05$. The spleen iron concentrations (Table 7)

TABLE 5. Hemoglobin (g/100 mL ± SD), hematocrit (% ± SD) levels, and hemoglobin–hematocrit ratios (mean of six to nine samples).

Group no.	Period 8 Hemoglobin	8 Hematocrit	8 Hg:HT	11 Hemoglobin	11 Hematocrit	11 Hg:HT
1	6.6±2.9	35±9	0.19	4.3±2.3	32±10	0.13
2	9.1±0.9	45±7	0.20	7.7±1.0	40±4	0.19
3	9.2±0.9	45±6	0.20	7.5±1.5	42±6	0.18
4	7.7±0.8	47±6	0.17	7.9±0.9	41±8	0.19
5	8.7±0.8	45±5	0.19	7.2±0.8	40±5	0.18
6	8.7±1.7	44±4	0.19	8.1±1.1	44±7	0.18
7	9.1±0.6	46±5	0.20	8.3±0.8	41±3	0.20
8	9.1±1.1	46±4	0.20	9.1±1.4	44±2	0.21

decreased with increasing ascorbic acid levels, with values for the highest spleen iron level being found in group 1. The decreases from group 2 through 8 were less marked but showed a definite downward trend; however, this regression was not statistically significant.

Discussion

The significant difference in the weight gain and feed efficiency of group 1 (Table 1) and the occurrence of deficiency symptoms in groups 1 and 2 indicate that supplemental ascorbic acid is required in practical trout diets. On the basis of the weight-gain response, feed–gain ratio, absence of deficiency symptoms, and tissue ascorbate concentrations, the level of supplementation in group 3 (40 mg/kg) appears to represent about the requirement, since the basal diet without supplemental ascorbic acid contained virtually no measurable concentration of this vitamin. However, previous studies have shown (Hilton et al. 1977) that the processing method employed in this study caused essentially the complete destruction of supplemental ascorbic acid at the level of 40 mg/kg in practical trout diets. This indicates that not only are the assay methods used not sufficiently sensitive to measure the small quantities of ascorbic acid remaining in these diets, but that the requirement of supplemental ascorbic acid in practical trout diets in this study was <40 mg/kg.

The NRC (1973) requirement for young rainbow trout fish is 3–5 mg·kg body $wt^{-1}\cdot d^{-1}$. With the upper level of 5 mg·kg body $wt^{-1}\cdot d^{-1}$, the actual level (milligrams per kilogram) of supplemental ascorbic acid required in the diet in order for the fish grown in this study to meet the NRC requirement was determined. The mean body weight per 100 fish was calculated in groups 3–8 for periods 1, 6, and 11 and the ascorbic acid requirement determined by multiplying this weight by 5 mg/kg body wt. The resulting number, the requirement per day (Table 8), was multiplied by 28, the number of days in a period, and then divided by the amount of food in kilograms consumed by the fish during that period. The requirement based on the NRC levels increases from 5.6 mg·kg body $wt^{-1}\cdot d^{-1}$ during period 1 to 172 mg·kg body $wt^{-1}\cdot d^{-1}$ during period 11 (Table 8). This would necessitate that the ascorbic acid concentration in the feed be in excess of 300 mg/kg during period 6 and 700 mg/kg during period 11. Supplemental ascorbic acid is very unstable in practical trout diets (Hilton et al. 1977) and it is therefore unlikely that many practical trout diets on the market today, unless kept refrigerated, would have or could maintain levels in excess of 300 mg/kg and certainly not 700 mg/kg for any length of time. Since instances of ascorbic acid deficiency symptoms in trout hatcheries are not common, we concluded that 3–5 mg·kg body $wt^{-1}\cdot d^{-1}$ for trout of the size used in this study is in excess of the actual requirement for larger fish at least.

In contrast to the results calculated by using the NRC recommendations, the requirement based on the minimum supplemental ascorbic acid level (40 mg/kg), which produced no deficiency symptoms in this study, decreased from 1.2 mg·kg body $wt^{-1}\cdot d^{-1}$ during period 1 to 0.3 mg·kg body $wt^{-1}\cdot d^{-1}$ during period 11 (Table 8). The discrepancy between the NRC recommendation and the experimental value found in this study may be partially explained by the difference in the age and genetic makeup of the trout used. Halver et al. (1975) suggested that the ascorbic acid requirement may change with the age and presumably the genetic strain of the trout. In the original studies (Halver et al. 1969), the initial body weight of the fry was 0.3 g with a final weight of 10 g. The present study involved trout that had an initial weight of 6.7 g (Table 1) and were approximately 18 wk old (time after hatching). Therefore, the two studies were different not only in type of diets used (semipurified vs. practical) but in size and age of trout used and probably genetic strain.

TABLE 8. Ascorbic acid requirements of rainbow trout based on the NRC requirement (3–5 mg·kg body $wt^{-1}\cdot day^{-1}$ 1973) and the supplemental level determined in this study.

	Period		
	1	6	11
Mean body wt (kg/100 fish)	1.1±0.2	12.4±0.6	34.3±0.8
Total feed consumed (g·100 $fish^{-1}\cdot 28\ d^{-1}$)	926±4	5524±260	6619±566
Requirement			
This study (mg·kg body $wt^{-1}\cdot d^{-1}$, assuming 40 mg/kg feed suppl. level is best approx.)	1.2±0.1	0.6±0.03	0.3±0.01
NRC (mg·kg body $wt^{-1}\cdot d^{-1}$)	5.6±0.1	61.8±3.2	171.7±4.2
Supplemental level (mg·kg)			
This study	40	40	40
NRC	169±3	313±8	728±49

The results suggest then that the ascorbic acid requirements of rainbow trout do change, being high (3–5 mg·kg body $wt^{-1}\cdot d^{-1}$) when the trout are young and decreasing as the fish grow. Halver et al. (1975) suggested that rainbow trout can store significant quantities of ascorbic acid. The trout in this study were fed a control diet containing in excess of 200 mg/kg (measured) for 4 wk previous to the start of the experiment. It is possible then that one or two other groups (3 and 4) were marginally deficient in the early stages of the experiment, but that these groups had sufficient stores of ascorbic acid to carry them over until the dietary level met their requirements. Also, note that the time period for storage of diets in this study varied between 5 and 8 wk at room temperature and it is conceivable that longer periods could markedly increase the apparent requirement of 40 mg/kg. In this connection we noted (Tables 3 and 4) that liver and head kidney ascorbic acid concentrations declined during period 11. It was during periods 10 and 11 that the storage period of the

diets reached the maximum of 8 wk. Therefore, the diets during this period would be expected to contain the lowest residual levels of ascorbic acid. The reduction in liver and head kidney ascorbic acid concentrations at the end of period 11 (Tables 3, 4) is probably related to this longer storage period.

It is difficult, on the basis of this study, to suggest a level of ascorbic acid to be included in a practical diet. The processing methods, moisture content of the feeds (during and after processing), length and type of storage conditions, and presence of environmental contaminants (chlorinated hydrocarbons and heavy metals) in feeds vary greatly. The level of supplemental ascorbic acid to be added to practical trout diets depends upon an evaluation of these factors, and a generally applied level of supplemental ascorbic acid does not appear to be feasible at this time.

The gradual lowering of hemoglobin and hematocrit levels (Table 5) indicates that scorbutic trout progressively develop an anemic condition. Although the hemoglobin–hematocrit ratio drops (Table 5), it is difficult to state what type of anemia is present. Only 80% of the fish in group 1 exhibited overt deficiency symptoms and random sampling techniques resulted in normal appearing as well as overtly scorbutic fish being analyzed. We noted that the normal appearing fish of group 1 had much higher hemoglobin and hematocrit levels than the scorbutic trout and this accounts for the variability noted in the results (Table 5). Therefore, it is not possible to state whether this anemia is microcytic or macrocytic without more research. The cause of the anemia and its relationship to ascorbic acid depletion are speculative. While the serum iron levels were lower or reduced in group 1 in comparison with the other groups (Table 6), there is no indication that this in itself would limit erythropoiesis. Bothwell et al. (1964) noted that although the plasma iron in scorbutic guinea pigs and siderotic Bantus with scurvy were lower than normal, this did not appear to limit the erythropoietic response. The reduced serum iron levels could well indicate an impaired release of iron from reticuloendothelial stores. Lipschitz et al. (1971) noted that ascorbic acid deprived guinea pigs had higher spleen iron levels than the controls, suggesting an impairment of the release of iron from reticuloendothelial cells of this organ. Spleen iron levels in this study (Table 7) were noted to be much higher in the scorbutic fish of group 1 than in the other groups. These results suggest that, as in other animals, ascorbic acid functions in iron metabolism, specifically in the release of iron from reticuloendothelial stores.

The liver iron levels (Table 7) indicate a direct relationship to dietary ascorbic acid levels. All three periods (6, 7, 10) showed significant ($P \leq 0.05$) regressions of liver iron concentration upon the dietary level of supplemental ascorbic acid. Fox (1975) found that ascorbic acid increased the intestinal absorption of iron in mammals. Therefore ascorbic acid may have caused an increase in the efficiency of intestinal iron absorption in rainbow trout. However, the larger spleen iron concentration of group 1 and the gradual reduction of spleen iron levels from group 2 to 8 suggest that, as in other animals (Lynch et al. 1974), ascorbic acid may also be effecting a redistribution of iron stores.

In summary, ascorbic acid is an essential vitamin for rainbow trout fed on a practical diet; however, an accurate quantitation of the requirement is dependent upon the evaluation of several factors, such as type of diet, processing, moisture content of the diet, storage time of the diet, and environmental temperature and humidity, level of environmental toxicants, and possibly the size, age, and genetic makeup of the trout. Furthermore, Halver (1972) indicated that the ascorbic acid requirement may be increased for wound healing, which may have practical implications. Under the conditions employed in this study 40 mg/kg of supplemental ascorbic acid before processing would appear to be sufficient to meet the requirements. Supplementation of practical trout diets should, however, be considerably in excess of this level due to the instability of ascorbic acid. The actual level of supplementation is also dependent on factors mentioned previously and therefore it is not possible to state a level of supplementation that will have general application over a wide range of conditions.

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pH-Induced Changes in Blood Lead of Lead-Exposed Rainbow Trout (*Salmo gairdneri*)

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Blood of juvenile rainbow trout (*Salmo gairdneri*) exposed to lead in water showed increasing lead concentrations as pH of the test water decreased from 10.0 to 6.0. A decrease in pH by 1.0 unit from any reference pH resulted in an increase of blood lead by a factor of 2.1. Since sublethal lead toxicity is related to uptake, these results suggest that toxicity increases as pH decreases. Control experiments indicated that reactions of lead with inorganic constituents of the test water were complete within 3 h and that blood lead was at equilibrium with water lead within 48 h. Therefore, at the time of blood sampling in the pH experiment, both lead complexation processes in the exposure system, plus lead uptake and release from the blood, were at equilibrium.

Key words: pH, lead, toxicity, fish, *Salmo*, blood, equilibrium.

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Le sang de jeunes truites arc-en-ciel (*Salmo gairdneri*) exposées à du plomb dans l'eau montre des teneurs en plomb qui augmentent à mesure que le pH de l'eau des essais passe de 10.0 à 6.0. Une diminution de 1.0 unité dans le pH à partir d'un pH de référence quelconque entraîne une augmentation du plomb sanguin d'un facteur de 2.1. Comme la toxicité sublétale du plomb est fonction de l'absorption, ces résultats suggèrent que la toxicité augmente à mesure que le pH diminue. Des expériences de contrôle indiquent que les réactions du plomb avec les constituants inorganiques de l'eau des essais sont terminées en dedans de 3 h et que le plomb sanguin est en équilibre avec le plomb de l'eau en dedans de 48 h. Il en résulte qu'au moment de l'échantillonnage du sang dans l'expérience de pH, les processus de complexation du plomb dans le système d'exposition, ainsi que l'absorption du plomb et la mise en liberté par le sang sont en équilibre.

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THIS paper demonstrates that blood lead concentrations of rainbow trout (*Salmo gairdneri*) exposed to waterborne lead increase as the pH of the test water decreases.

Davies et al. (1976) and Holcombe et al. (1976) have shown that exposure of fish to sublethal concentrations of lead for periods of at least 3–6 mo caused symptoms of spinal deformities. Since mine drainage may depress pH as well as contribute metals to streams (Packer and Dunson 1970), the effect of pH on sublethal lead toxicity was studied.

Davies et al. (1976) and Hahne and Kroontje (1973) showed that the pH of water controls not only the type of lead complexes present but also their concentration. If it were known which complexes were available for uptake and toxicity, consideration of the chemistry of lead in water would allow the prediction of pH effects on sublethal toxicity without lengthy experiments. A recent workshop, however, indicated that routine measurements of the concentration of specific complexes of a given metal (e.g. $PbOH^+$) is not currently possible (IJC 1977). This approach also does not consider the effect of pH on the fish, on its permeability to lead, and on its reaction to lead. Therefore, pH effects on sublethal lead toxicity cannot be predicted chemically.

If it is assumed that only lead taken up exerts a chronically toxic effect, variations of lead uptake should indicate how sublethal toxicity will vary with pH. Increasing lead concentrations in water cause proportionate increases of blood lead concentrations and sublethal toxic effects in fish (Holcombe et al. 1976; Hodson et al. 1977). In mammals, there is also a strong correlation between blood lead and toxicity (Chisholm 1973) and blood lead is considered the best indicator of a harmful lead exposure. Therefore, the purpose of our first experiment was to estimate pH effects on sublethal lead toxicity to fish by measuring blood lead.

Lead added to test water as a dissolved concentrated stock in acidified, distilled water will form the various complexes after mixing. Consequently, availability of lead to the fish may change with time as these reactions occur, and the pH effects may be confounded. Therefore,

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a second experiment was conducted to test the effect of time for these reactions to take place on blood lead of exposed fish.

The lead content of fish blood should represent a dynamic equilibrium between lead uptake from water across the gills, and lead loss to other tissues. Since this equilibrium must be established if pH effects are to be measured without confounding, a third experiment tested the effect of exposure time on blood lead concentrations of fish.

Materials and Methods

Experiments were conducted with continuous-flow exposure systems, based on a modified proportional diluter (Mount and Brungs 1967) and a doser driven by an intermittent siphon bottle (Hodson 1974). The dilution water was from Lake Ontario and has been described by Hodson et al. (1977). All experiments were conducted at 15°C with dissolved oxygen close to saturation.

The test species was rainbow trout (*Salmo gairdneri*) obtained as fingerlings. They were maintained at 15°C for several months before use and fed once daily, ad lib., with a dry pelletized commercial trout food. Since each experiment was conducted separately, average fish size was not constant between experiments.

Blood lead concentration and erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) activity, an indicator of blood lead concentrations, were measured according to the methods of Hodson et al. (1977). Results were analyzed by an analysis of variance appropriate to each experiment (Steel and Torrie 1960). Statistical significance of difference between means was tested at the 95% confidence level by partitioning the treatment sums of squares and computing a separate "*F*" value for each comparison.

pH Effects on Blood Lead

Four-month-old rainbow trout averaging 15 g each were exposed in groups of five to nominal concentrations of 0 (control), 32, 56, 100, 180, 320, 560 and 1000 $\mu g \cdot L^{-1}$ lead at nominal pHs of 6, 8, and 10 for 3 d in one experiment and 7, 8, and 9 for 2 d in a second experiment. The distribution of the treatments among tanks was randomized as was the order in which fish were sampled for blood lead analyses. Fish were not fed during these brief exposures. Every 2½ min, the diluter delivered 2 L of solution per concentration split three ways to give about 270 $mL \cdot min^{-1} \cdot tank^{-1}$, or 5 $L \cdot g$ of $fish^{-1} \cdot day^{-1}$.

A 1% solution (vol/vol) of H_2SO_4 (.21 $mol \cdot L^{-1}$) was dripped from a constant head reservoir into one of the three outlets of the splitter cells to give a low pH at each lead concentration. Similarly, a 1% NaOH solution (wt/vol; .25 $mol \cdot L^{-1}$) was added to a second outlet of the splitter cells to give a high pH at each concentration. The third outlet was left untreated at pH 8.0, the natural pH of the dilution water. The amount of acid and base required for the extremes of pH was about 3.5 and 3.0 $mL \cdot L^{-1}$ of water, respectively. However, fine control of pH was achieved empirically by adjusting the drip rates until the pH in each tank was at the desired level. The pH was measured in each tank 2–3 times daily while lead was measured once daily by atomic absorption spectrophotometry (AAS). Results of the blood lead measurements were transformed to natural logarithms before statistical analysis.

Effect of Reaction Time on Blood Lead

Fish averaging 53 g each were exposed at pH 8 to a nominal concentration of 100 $\mu g \cdot L^{-1}$ lead in tanks containing either 27, 42, 63, or 155 L of water. Flow to each tank was 340 $mL \cdot min^{-1} \cdot tank^{-1}$ or about 1.85 $L \cdot g$ of $fish^{-1} \cdot day^{-1}$. The combination of a constant flow rate and increasing tank volumes gave 95% molecular replacement times of 5.4, 9.5, 15.5, and 34 h, respectively (Sprague 1969). The net result was an increasing time for chemical reactions between lead and the constituents of the test water. Each combination of lead and reaction time was duplicated for a total of 8 tanks plus 2 control tanks at a replacement time of 5.4 h. The experiment was repeated a second time. Five fish were added to each tank, and, after 1-wk exposure, blood was sampled from 3 fish per tank for a total of 60 sampled fish.

To determine whether reaction times were concentration dependent, a second experiment at pH 8 was conducted with three trout per tank averaging 121 g each. The 95% molecular replacement times were adjusted, as described above, to 3, 12, and 21 h at a flow rate of 600 $mL \cdot min^{-1} \cdot tank^{-1}$. The flow equalled about 2.4 $L \cdot g$ of $fish^{-1} \cdot day^{-1}$. Nominal lead concentrations were 0 (control), 100, 180, 320, 560, and 1000 $\mu g \cdot L^{-1}$ giving a 6 concentration × 3 turnover time experiment. Fish were fed during the exposure at a rate of 0.5% of their body weight per day. After 1-wk exposure, blood samples were taken for lead analysis and assay of ALA-D activity.

In both of the above experiments, lead concentrations were measured daily by AAS in the water of exposure tanks to determine the true exposure concentrations.

Effect of Exposure Time on Blood Lead Content

Rainbow trout averaging 15 g each were exposed at 15°C, pH 8, to nominal concentrations of 100 and 1000 $\mu g \cdot L^{-1}$ lead in three groups of six fish at each concentration. Flow rates to each tank were the same as in the pH experiment. One fish per tank was sampled at 0, 2, 4, 8, 24, and 48 h from the start of exposure and a blood sample from each fish was analyzed for erythrocyte ALA-D activity and blood lead concentration.

Results

pH Effects on Blood Lead

Mean measured pHs deviated by less than 8, 3, 1, 1, and 3% from nominal levels at pHs of 6, 7, 8, 9, and 10, respectively, except in two cases at pH 6 and one case at pH 9 (Table 1). In the worst case, at pH 4.85, all fish died and there were no results. Variability of pH tended to increase as pH was adjusted away from 8. Lead concentrations in water at pH 6, 7, and 8 were similar and results of multiple analyses at 100 and 1000 $\mu g \cdot L^{-1}$ indicated good uniformity, with coefficients of variation of 6–12%. At pH 9 and 10, however, variability increased (coefficients of variation = 25–30%) and total lead concentrations decreased from those at pH 8 by 0–19% and 13–34%, respectively. A fine white precipitate was observed closely adhered to the tank walls but analyses of lead were probably not affected since the precipitate failed to contaminate clean water after resuspension and settling.

Mortality of exposed fish appeared to be related to extremes of pH rather than to lead concentration (Table 1).

TABLE 1. Means of pH and total lead measurements of test water plus percent mortality of exposed fish (five per tank). The numbers of pH observations per tank for pH 6, 7, 8, 9, and 10 were 8, 6, 5, 6, and 6, respectively. The numbers of lead observations at these pHs were 2, 1, 2, 1, and 2, respectively, except at nominal concentrations of 100 and 1000 $\mu g \cdot L^{-1}$ where N was 6–11. Standard deviation for these two lead concentrations are given in parentheses.

	Nominal lead concn ($\mu g \cdot L^{-1}$)							
Nominal pH	0 (Control)	32	56	100	180	320	560	1000
6.0 pH								
$\bar{x}$	6.05	5.92	6.23	4.85	6.13	6.46	5.96	6.86
SE	.18	.17	.72	1.62	.36	.79	.21	1.11
range	5.71–6.18	5.69–6.13	5.22–7.70	2.86–6.34	5.63–6.50	5.55–7.55	5.61–6.32	4.96–8.12
lead ($\mu g \cdot L^{-1}$)	10	64	70	120 (14)	200	290	500	920 (60)
percent mortality	0	0	80	100	0	20	20	80
7.0 pH								
$\bar{x}$	6.79	6.93	7.22	6.91	7.11	7.05	6.80	6.88
SE	.06	.15	.27	.12	.53	.10	.06	.13
range	6.69–6.88	6.65–7.07	7.06–7.77	6.80–7.14	6.83–8.18	6.91–7.06	6.72–6.88	6.72–7.1
lead ($\mu g \cdot L^{-1}$)	6	80	—	120	—	—	490	900
percent mortality	20	0	0	20	40	0	0	60
8.0[a] pH								
$\bar{x}$	7.97	8.04	7.97	7.98	8.00	7.96	7.98	7.95
SE	.06	.15	.08	.10	.13	.07	.10	.19
range	7.86–8.00	7.86–8.22	7.83–8.01	7.88–8.15	7.86–8.19	7.85–8.03	7.83–8.14	7.61–8.12
lead ($\mu g \cdot L^{-1}$)	5	52	85	120 (10)	210	300	500	910 (60)
percent mortality	10	10	10	0	0	0	20	10
9.0 pH								
$\bar{x}$	8.93	8.53	9.08	8.76	9.06	9.06	8.93	9.04
SE	.10	.38	.07	.47	.23	.06	.54	.04
range	8.79–9.03	8.16–9.04	8.96–9.13	8.06–9.15	8.60–9.19	9.01–9.18	8.07–9.41	8.99–9.10
lead ($\mu g \cdot L^{-1}$)	4	53	—	110	—	—	470	740
percent mortality	0	0	0	0	20	0	0	0
10.0 pH								
$\bar{x}$	10.06	9.71	10.13	9.89	10.00	9.88	9.96	10.01
SE	.15	.15	.08	.05	.06	.09	.12	.11
range	9.81–10.22	9.53–9.92	10.03–10.22	9.83–9.94	9.94–10.07	9.78–10.04	9.74–10.12	9.86–10.10
lead ($\mu g \cdot L^{-1}$)	3	40	57	92 (28)	140	210	300	790 (200)
percent mortality	100	60	100	100	100	80	60	100

[a]All measurements at pH 8 represent the means of results across two tests (one at pH 6, 8, and 10, the second at pH 7, 8, and 9).

Overall mortality at pH 6, 7, 8, 9, and 10 was 38, 18, 8, 3, and 88%, respectively. Consequently there was very little data at pH 10.

The blood lead concentration of trout increased with an increase in water lead concentration and a decrease in water pH (Fig. 1). The slopes of the lines relating lead in water to lead in blood appear similar although there may be a plateau at pH 6. An analysis of variance indicated significant variation of blood lead with pH and lead in water, with a slight but significant pH × lead interaction, probably due to the results at pH 6. The pH effect on blood lead was very clear and uniform (Fig. 2) with increasing pH causing large decreases in blood lead. The slopes of all lines of Fig. 2 (except control) appear parallel, i.e. there was a similar relative change in blood lead for a given change in pH, regardless of lead concentration in water. Consequently, for each lead concentration in water, blood lead at each pH was expressed as a proportion of blood lead at pH 8 since this was the normal pH of the test water. The result was a population of relative blood lead values at each pH (Fig. 3). The mean relative blood lead values fall on a straight line with the exception of pH 10 for which there may be too little data. Despite the apparent variability, an analysis of variance of these data (excluding data at pH 8, the reference point) demonstrated that relative blood lead varied significantly with pH. A decrease in water pH of 1.0 U approximately doubled blood lead (slope = 2.1).

EFFECTS OF REACTION TIME ON BLOOD LEAD

Increasing the time for 95% molecular replacement of water containing 100 $\mu g \cdot L^{-1}$ nominal lead from 5.4 to 34 h had no significant effect on measured total lead concentrations in water, nor on blood lead and erythrocyte ALA-D activity of exposed fish (Fig. 4). The same

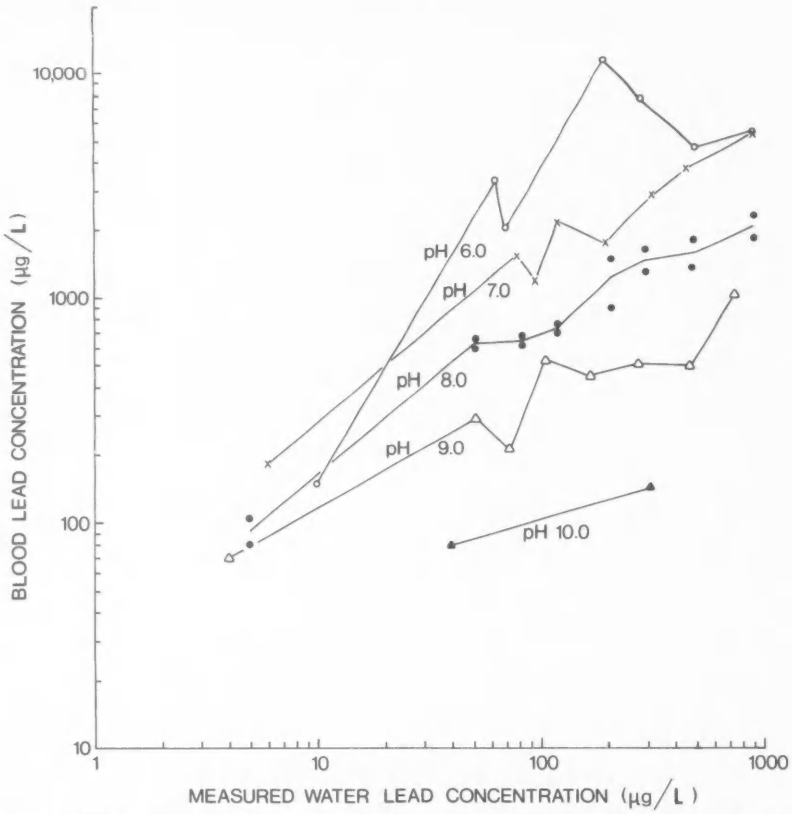

FIG. 1. Relationship between waterborne lead concentration and blood lead concentration of rainbow trout at several pHs. Data at waterborne lead concentrations less than 10 $\mu g \cdot L^{-1}$ represent control fish. Each point represents five fish unless mortality reduced the sample size. Mortality is given in Table 1.

result was also seen in a second experiment at nominal lead concentrations up to 1000 $\mu g \cdot L^{-1}$. A small amount of white precipitate was observed at 1000 $\mu g \cdot L^{-1}$ lead. There was 25% mortality of exposed fish with all deaths at 560 and 1000 $\mu g \cdot L^{-1}$ lead. The toxicity of lead (number of deaths) did not vary with the 95% molecular replacement time.

EFFECTS OF EXPOSURE TIME ON BLOOD LEAD

The means and standard deviations of measured lead concentrations in exposure tanks were 6 ± 1, 130 ± 21, and 900 ± 69 $\mu g \cdot L^{-1}$ at nominal concentrations of 0 (control), 100, and 1000 $\mu g \cdot L^{-1}$, respectively. Lead-exposed fish accumulated lead rapidly so that, by 48 h, curves of blood lead concentration and erythrocyte ALA-D activity had leveled off (Fig. 5). An analysis of variance at the 95% confidence level showed that these parameters varied significantly with time, but that there was no significant difference between means at 24 and 48 h.

Discussion

These results clearly demonstrated that pH of the test water controls lead uptake by rainbow trout and that the test systems used were in equilibrium with respect to availability of lead and lead concentrations in blood of exposed fish.

The pH effect on lead uptake by fish may be attributed to a change in the concentrations of lead complexes available to fish, a change in the permeability of the fish, or a combination of these changes.

Precipitation of lead at pH 9 and 10 obviously reduced total lead available to the exposed fish, but at pHs less than 9 no lead precipitated and changes in the proportions of various lead complexes are unknown. If decreased pH affects blood lead by increasing the con-

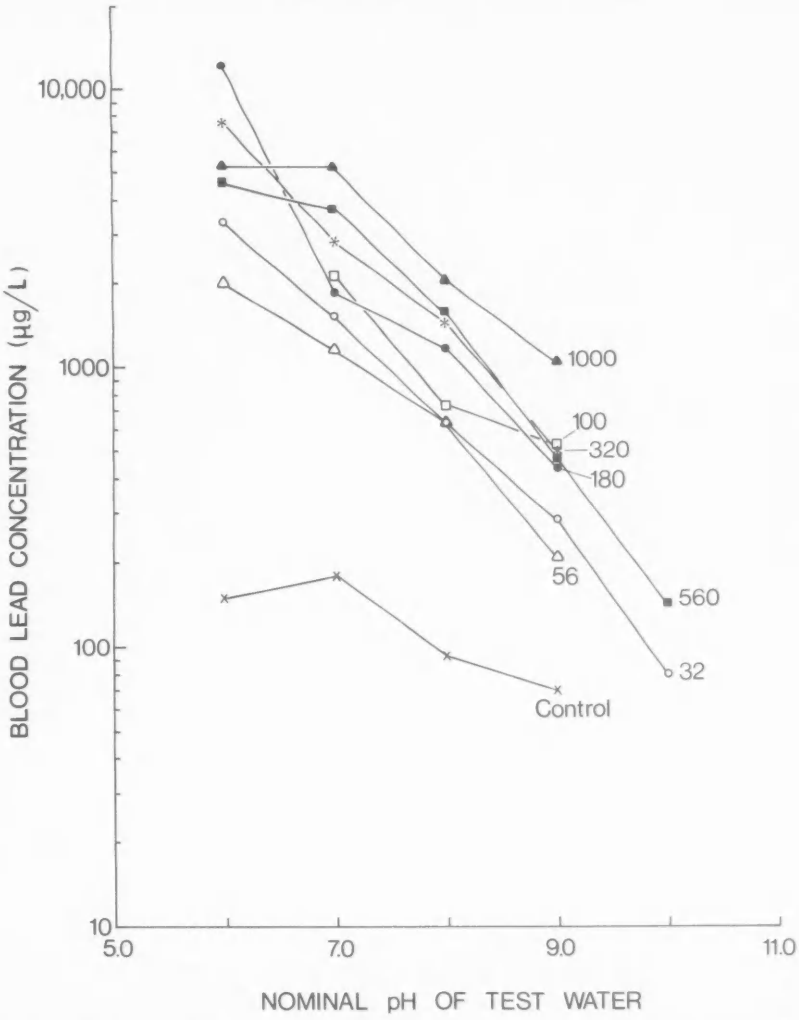

FIG. 2. Effect of pH of test water on blood lead concentrations of rainbow trout exposed to waterborne lead. Numbers on the right refer to the nominal concentrations of waterborne lead ($\mu g \cdot L^{-1}$).

centration of available lead complexes (i.e. available lead in water becomes a greater proportion of total lead), then the concentration of these complexes should increase with decreasing pH at the same rate as did blood lead. The solubility diagrams presented by Davies et al. (1976) indicate that a drop in pH from 8.0 to 7.0 increases the concentrations of ionic lead (Pb^{2+}) and a variety of other lead complexes by a factor of about 100 in soft water and 150 in hard water. However, this pH drop increased blood lead of trout by a factor of 2.1. Therefore, pH effects on blood lead and pH effects on solubility of lead complexes do not correspond. These solubility diagrams are theoretical and based on measurements at 25°C in an artificial water free from organic material. Therefore, complete evaluation of the first and third alternative explanations of pH effects on blood lead depends on a better understanding of lead chemistry in natural waters.

The second alternative, that pH effects are a result of changes in membrane permeability of the fish, is supported by consideration of the physiological effects on fish of reduced pH. Packer and Dunson (1970, 1972)

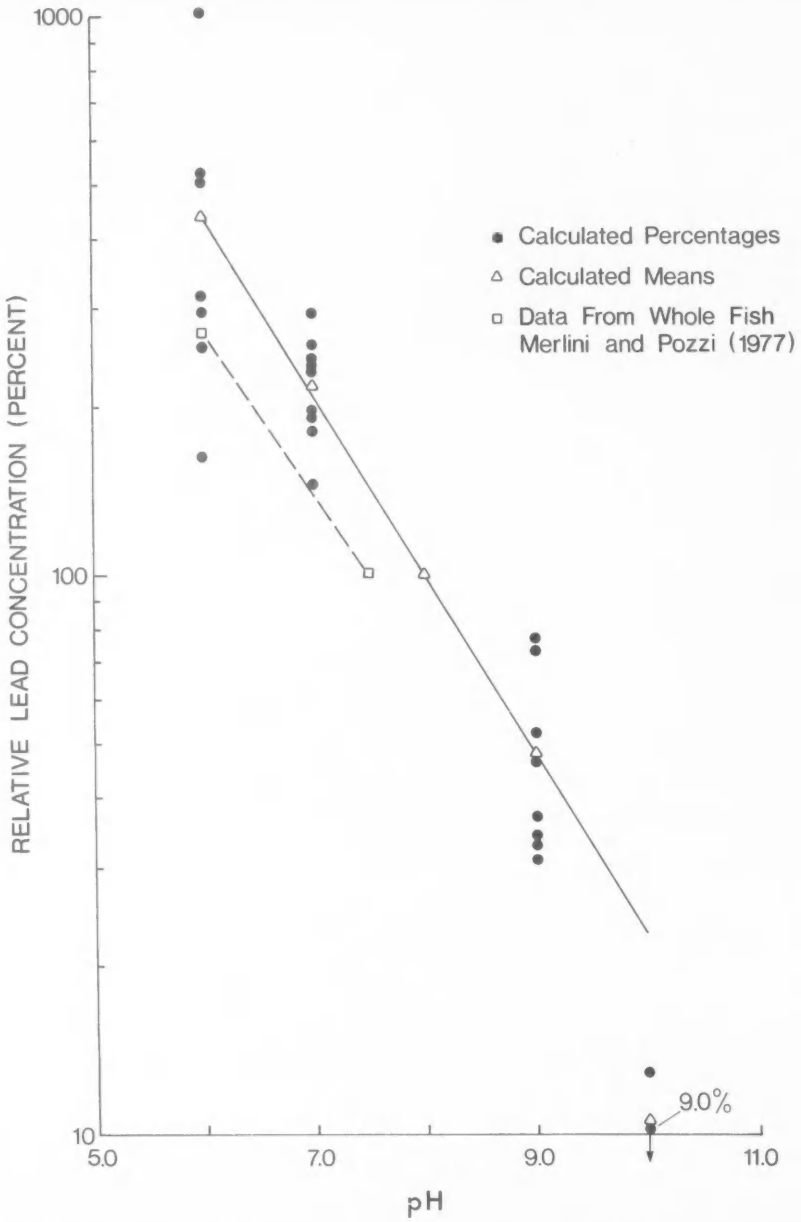

FIG. 3. Effect of pH of test water on relative blood lead concentrations of rainbow trout exposed to waterborne lead. For any lead concentration in water, relative blood lead was calculated as the ratio of blood lead at a test pH to the blood lead at pH 8.0 ($\times 100$ for %). Dashed line represents data for whole sunfish (*Lepomis gibbosus*) exposed to lead at pH 7.5 and 6.0 by Merlini and Pozzi (1977). Lead content of fish at pH 6.0 was expressed as a ratio of lead content at pH 7.5 ($\times 100$ for %). Solid line may be described by the equation: relative blood lead = $100 \times (2.1^{-\Delta pH})$ where ΔpH is the difference between a test and a reference pH (in this case 8.0).

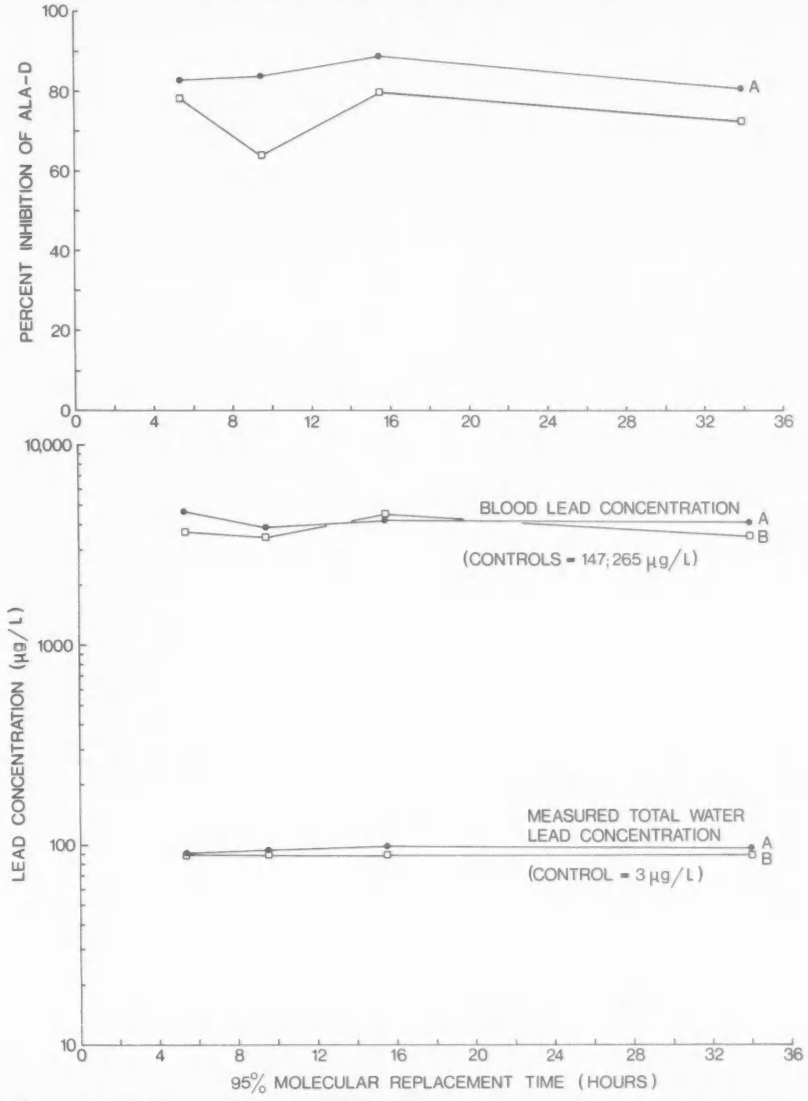

FIG. 4. Effect of increasing time for 95% molecular replacement of water in exposure tanks on concentrations of lead added to water, blood lead of exposed rainbow trout, and inhibition of erythrocyte ALA-D of the same trout. Control water contained 3 $\mu g \cdot L^{-1}$ lead and blood lead of control fish was 147 and 265 $\mu g \cdot L^{-1}$ in the first (A) and second (B) trials, respectively. Control ALA-D activity was taken as 0% inhibition.

reported a greatly increased sodium loss from brook trout exposed to low pH. This was possibly a result of increased ion exchange activity (either in gills or kidney) as the fish attempted to regulate H^+ ions, or a direct change in gill membrane structure and permeability. Packer and Dunson (1970) also showed that low environmental pH decreased blood pH. Since this caused decreased oxygen carrying capacity of blood (due to Bohr and Root effects on hemoglobin, Prosser and Brown 1961) the brook trout showed signs of respiratory stress. In this experiment, a nonlethal reduction of blood pH would increase respiratory water and blood flow as the fish attempted to compensate for decreased O_2 carrying capacity of the blood. The net result would be increased availability of oxygen and all other dissolved constituents of water including lead. Therefore, pH could

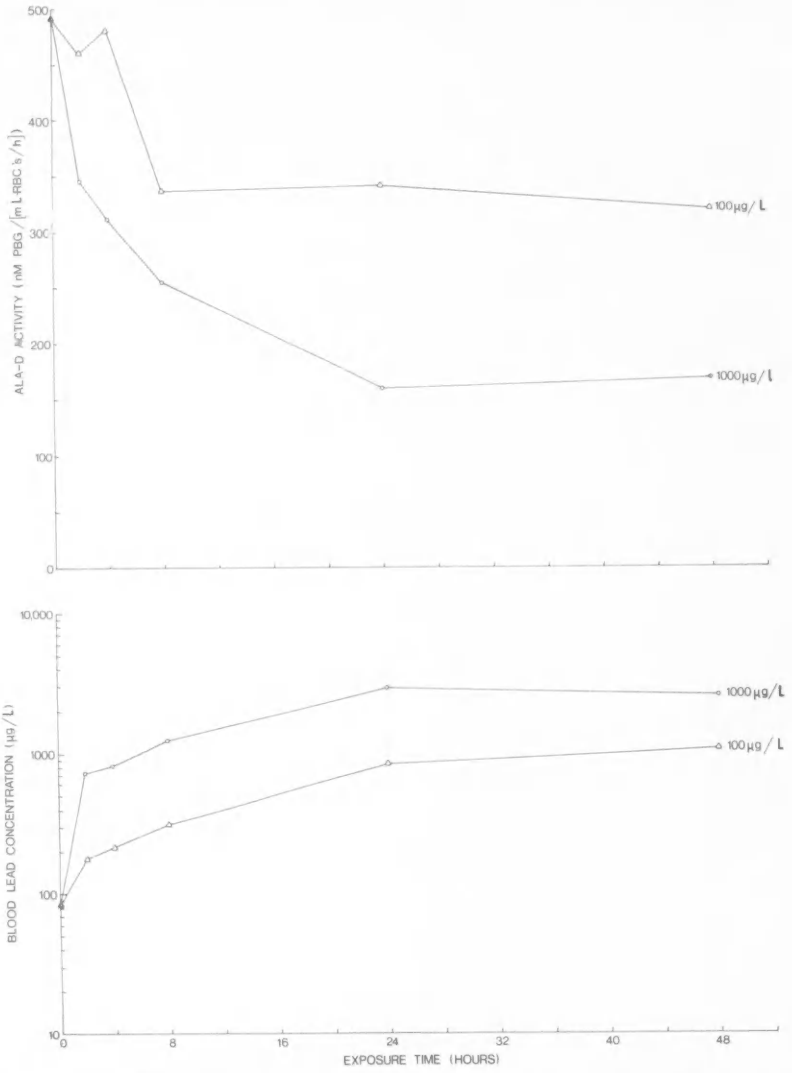

FIG. 5. Effect of exposure time on blood lead content and erythrocyte ALA-D activity of rainbow trout exposed to 100 and 1000 $\mu g \cdot L^{-1}$ lead in water.

also have influenced lead uptake by both direct and indirect effects on permeability.

These results correspond closely to those of Merlini and Pozzi (1977) who exposed sunfish (*Lepomis gibbosus*) at 19°C to 40 $\mu g \cdot L^{-1}$ stable lead, plus 222 kBq $\cdot L^{-1}$ (6 $\mu Ci \cdot L^{-1}$) of ^{203}Pb as a tracer, at pHs of 6.0 and 7.5. Lead concentrations were measured in whole fish and in various tissues at 2, 4, 6, and 8 days and the ratio of lead concentrations at pH 6.0 to lead at pH 7.5 calculated. The average ratio was 2.70:1 or an increase of lead residues by 1.95 times for every decrease of pH by 1.0 U (Fig. 3). This corresponds quite well to the factor of about 2.1 observed in this experiment. The water used by Merlini and Pozzi (1977) was very soft (calcium and magnesium equalled 15 and 4 mg/L, respectively), so that 40 $\mu g \cdot L^{-1}$ of lead was well within the "solubility" limits of 500 $\mu g \cdot L^{-1}$ at this hardness and these pHs (as defined by pulse polarography, Davies et al. 1976). Since lead toxicity is closely related to "soluble" lead (Davies et al. 1976) and since all the lead was "soluble," the effects of low pH were most likely changes of permeability of the sunfish rather than of lead chemistry.

The effect of pH on lead uptake by fish is quite important since low pH is a common property of many industrial effluents and of soft waters, especially those receiving acidic fallout from air pollution. If toxicity can be directly related to blood lead concentrations, as suggested in the introduction, then water quality objectives for lead should be adjusted for pH; i.e. when pH decreases by 1.0 U, the objective should be decreased by $\frac{1}{2}$. Also, if a major effect of low pH is on the permeability of fish gills, rather than on solubility of the metal, then objectives for other metals may have to be adjusted for pH.

One drawback to both experiments was that fish were transferred instantaneously to a new pH without opportunity for acclimation. In this experiment, the result was mortality at extremes of pH. Since salmonids are known to survive pH 10.0 in the short-term and pH 6.0 in the long-term (NAS/NAE 1974), acclimation should be possible. If acclimation reduces the detrimental effects of pH, then it may also influence lead uptake. Therefore, these results are most indicative of effects on fish encountering a sudden change in pH. A possible cause of the mortality at pH 6 is free CO_2. However, aeration within distribution tubes would help reduce free CO_2 prior to the exposure tanks.

The results of the chemical equilibrium tests indicated that chemical reactions rendering lead unavailable for fish occur in less than 3 h. Reaction times greater than 3 h, tested by increasing the time to 95% molecular replacement, did not reduce lead available to fish for uptake. Consequently, these tests of lead in laboratory flowing-water systems were conducted in a situation of constant availability of metal.

The experiment testing the effect of exposure time on blood lead of rainbow trout indicated that blood lead reached an equilibrium with water very quickly. While changes in concentration may occur over a much longer time scale in response to changing environmental conditions, short-term comparisons (e.g. between pHs) may be made without confounding. Holcombe et al. (1976) showed that blood lead of brook trout increased rapidly during lead exposure after which large fluctuations occurred. Blood lead was first tested after 4 wk exposure and the subsequent fluctuations were not well explained. They were probably a response to fluctuating lead concentrations in water as proposed by Hodson et al. (1977).

Inhibition of ALA-D followed the same time scale as lead concentration in blood. This means that lead taken up is available immediately for toxic effects in the blood and this toxicity is limited only by uptake dynamics. These results are similar to those of Hodson (1976) and Hodson et al. (1977) who observed that, after 1 wk of exposure (the minimum exposure time), ALA-D inhibition by lead showed no consistent trends in time.

Acknowledgments

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Changes in the Predator–Prey Behavior of Fathead Minnows (*Pimephales promelas*) and Largemouth Bass (*Micropterus salmoides*) Caused by Cadmium

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SULLIVAN, J. F., G. J. ATCHISON, D. J. KOLAR, AND A. W. MCINTOSH. 1978. Changes in the predator–prey behavior of fathead minnows (*Pimephales promelas*) and largemouth bass (*Micropterus salmoides*) caused by cadmium. J. Fish. Res. Board Can. 35: 446–451.

Increased prey vulnerability was demonstrated for fathead minnows (*Pimephales promelas*) undergoing acute (24-h) and subacute (21-d) sublethal cadmium exposure prior to interacting with largemouth bass (*Micropterus salmoides*). The lowest acute and subacute cadmium concentrations that increased prey vulnerability were 0.375 and 0.025 mg Cd/L, respectively, with the latter well below the maximum acceptable toxicant concentration for fathead minnows. Prey exposed to cadmium displayed altered behavior patterns, including abnormal schooling behavior.

Key words: cadmium, behavior, predator–prey, bioassay, *Micropterus salmoides*, *Pimephales promelas*

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Les têtes-de-boule (*Pimephales promelas*) subissant une exposition au cadmium sublétale aiguë (24 h) et subaiguë (21 jours) avant interaction avec des achigans à grande bouche (*Micropterus salmoides*) montrent une vulnérabilité accrue comme proies. Les concentrations aiguës et subaiguës de cadmium les plus basses entraînant une augmentation de vulnérabilité sont de 0.375 et 0.025 mg de Cd/L respectivement, cette dernière concentration étant de beaucoup inférieure à la concentration acceptable maximale du toxique pour les têtes-de-boule. Les proies exposées au cadmium montrent des comportements différents, y compris un comportement anormal de formation de bancs.

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STUDIES investigating the effects of sublethal levels of pollutants on the behavior of aquatic organisms are generating information and ideas that are timely additions to the classical bioassay literature. A number of environmental stresses have been shown to alter the predator–prey interactions between various species of fish, presumably through alteration of prey-fish behavior. These stresses include Sumithion, an organophosphate pesticide (Hatfield and Anderson 1972), mercury (Kania and O'Hara 1974), ionizing radiation (Goodyear 1972), and thermal stress (Sylvester 1973; Coutant 1973). The changes in prey-fish behavior, resulting in selective predation on the stressed prey, were most often recorded at levels of the stress that were well below lethal levels. Since typical predator–prey studies have not included actual behavioral observations of the test organisms, it is of interest to determine what behavioral alterations, if any, may result in decreased prey survival following exposure to sublethal levels of pollutants. Differences between treated and control fish behavior may be gross, and therefore obvious upon direct observation, or may occur at more subtle levels which are detectable only by other fish.

Coble (1973) found that the behavior of prey fish was more important than their physical appearance in influencing predator choice and thus prey survival. If changes occur in prey-fish behavior as a result of exposure to physiologically sublethal concentrations of toxicants, it follows that their vulnerability to predation may be changed. Therefore, sublethal exposure levels would be indirectly lethal to prey fish if predators selectively feed on them. Thus it is important to assess the effects of sublethal levels of pollutants on predator–prey interactions.

Studies utilizing acute exposure times are of interest for

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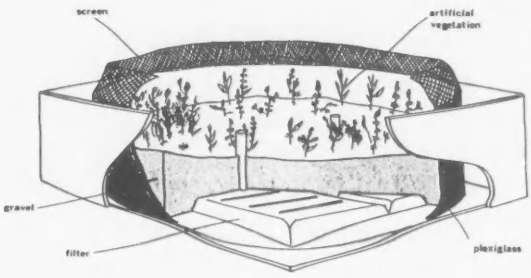

FIG. 1. Cross section of 122 × 122 × 41 cm experimental predator–prey chambers.

a number of reasons. Exposure to high concentrations of toxic substances for short periods of time, while not commonly encountered by fish in natural waters, does occur in the case of industrial spills. In addition, all predator–prey studies reported in the literature have utilized acute exposures. However, as a result of dilution and dispersal of pollutants in the aquatic environment, a much greater proportion of biomass is chronically exposed to low, sublethal levels of toxic compounds than to acutely lethal levels (Kleerekoper 1976). Therefore, it is perhaps more important to investigate the effects of toxicant exposure for longer periods of time on predator–prey interactions than to observe changes in predation pressure after acute exposure.

The objectives of this study were the following: (1) to assess the impact of acute and subacute (21-d) Cd exposure on the vulnerability of fathead minnows (*Pimephales promelas*) to predation by largemouth bass (*Micropterus salmoides*); (2) to develop methods for studying the effects of pollutants on predator–prey interactions; and (3) to determine if recognizable alterations in prey-fish behavior do occur after exposure to a toxicant.

Materials and Methods

The yearling largemouth bass utilized as predators ranged from 18 to 21 cm long, with an average weight of 100 g. Fathead minnows were approximately 3.5 cm long and averaged 0.8 g. All largemouth bass and those minnows utilized for acute exposure tests were pond reared and obtained from Aquatic Control, Seymour, Indiana. The Newtown Fish Toxicology Laboratory, Cincinnati, Ohio, supplied the fathead minnows for the 21-d exposure tests. All minnows were maintained throughout the study on a diet of frozen brine shrimp.

The interiors of three experimental tanks (Fig. 1), 122 × 122 × 41 cm, were encircled with plexiglass to eliminate corners which could be used by predators to trap prey. The gravel covering the biological filters was graded to provide the deepest water in the center of the tank and shallower water at the edges. Artificial vegetation was planted throughout the tanks, with the plants heavily clustered in one quadrant. This design provided cover for the predator and the prey fishes. Blinds with an observation slit on one side were built around the tanks to limit disturbance of the fish by the observer. Tanks were maintained on a 16-h light : 8-h dark photoperiod with temperatures ranging from 19 to 21°C.

The bass were fed a maintenance diet of assorted forage fish until 20 h prior to placement into the experimental tanks. One bass was used per tank to eliminate variability resulting from social interactions. All minnows were marked by a liquid nitrogen cold-branding technique (Fujihara and Nakatani 1967) at least 14 d prior to initiation of a test. The controls were randomly branded on one side and the Cd-exposed fish on the opposite side. Postbranding mortality was <1%. All tests were run in triplicate, with 20 control and 20 treated fish per tank with the exception of the 500 μg/L acute exposure, in which 14 treated and 14 controls were used.

Forty-eight-hour static or 21-d continuous-flow treatments were utilized to sequentially expose the prey to various concentrations of Cd (Table 1), which was administered as analytical grade cadmium chloride, $CdCl_2 \cdot 2.5\ H_2O$. Each concentration-control test was performed in triplicate after one triplicate exposure of the minnows to either Cd-treated or control water. Control fish received the same treatment (minus the Cd) as did the Cd-exposed fish. Dechlorinated charcoal-filtered tap water was delivered to each continuous-flow exposure and control tank at a rate of 200 ± 10 mL/min. Replacement time for 95% of the test water was calculated to be 5.8 h. Cadmium stock solution was delivered via a four-channel, variable-speed peristaltic pump to a mixing flask where it was combined with dilution water and delivered by gravity flow to the test tanks. Minnows were fed a daily maintenance diet of freeze-dried brine shrimp during the 21-d exposure. To avoid precipitation of the Cd out of solution during 48-h static tests, exposure water of low hardness was prepared by mixing charcoal-filtered tap water and distilled water. Cadmium concentrations in the water were determined by atomic absorption spectrophotometry (Sullivan et al. 1977). Hardness, alkalinity, pH, dissolved oxygen, and temperature of the exposure water were routinely measured (Table 2) according to the methods of the APHA et al. (1971).

TABLE 1. The concentrations (mg/L) of cadmium utilized for 48-h and 21-d exposures. Theoretical Cd concentration was based on Cd added, volume of water, and/or flow rate. Actual Cd concentration are as determined by atomic absorption spectrophotometry.

48-h			21-d		
Theoretical	No. of analyses	Actual (1 SD)	Theoretical	No. of analyses	Actual (1 SD)
0.500	2	0.491 (0.010)	0.050	6	0.048 (0.001)
0.375	2	0.370 (0.002)	0.025	6	0.025 (0.002)
0.250	2	0.249 (0.006)	0.019	5	0.018 (0.001)
0.050	2	0.043 (0.006)	0.013	6	0.013 (0.001)

TABLE 2. Chemical and physical characteristics of exposure water.

Characteristic	48-h No. of analyses	48-h Mean (1 SD)	21-d No. of analyses	21-d Mean (1 SD)
Dissolved oxygen (mg/L)	8	8.8 (0.11)	30	8.1 (0.24)
pH	8	8.33 (0.09)	28	7.85 (0.18)
Hardness (mg/L $CaCO_3$)	8	41 (2.39)	30	349 (7.4)
Alkalinity (mg/L $CaCO_3$)	8	44.5 (1.77)	30	242 (6.0)
Temperature (°C)	8	21.3 (0.5)	30	15.6 (0.75)

Following Cd exposure, the minnows were held in clean water for 48 h to allow the prey to somewhat recover from the stress of Cd exposure and to minimize contamination of the experimental tanks prior to transfer to the experimental tanks. A 48-h acclimation period to the test tanks was allowed before adding the predator. Each bass was placed in the tank near the edge with minimal disturbance of the minnows. The interaction between the minnows and the bass was extensively observed until approximately 50% of the prey had been eaten (Bams 1967; Coutant 1973), at which time all surviving prey were removed. The number of control and treated minnows surviving was determined via brand identification. Surviving prey were not used in subsequent tests.

To observe individual and group behavior of the fish effectively, 300-L rectangular fiberglass tanks with a 112 × 46 cm viewing window and fitted with gravel and artificial vegetation were utilized. Fathead minnows were differentially branded prior to acute exposure to 0.45 mg Cd/L. Behavioral observations were taken for 3 h/d for 5 d after the addition of the largemouth bass to assess any changes in prey behavior elicited by exposure to the Cd. Brand identification allowed for easy, rapid differentiation between treated and control fish during these observation periods.

The statistics used in this study to analyze the difference in predation rates upon the two groups of fish were based on the ratio T/R, where T and R are the number of treated fish eaten and the total number of fish eaten in any particular run, respectively. To assess the precise effect (K) that a given Cd exposure level had on the vulnerability of treated fish to predation, a model of $P_t = KP_c$ was assumed, where $P_t(P_c)$ is the probability that a particular treated (control) fish will be eaten next. If there are m treated and n control fish in an experimental tank, then $P_t = K/(mK + n)$, $P_c = 1/(mK + n)$, and $mP_t + nP_c = 1$. Thus K is a measure of the change in prey vulnerability due to treatment with a toxicant.

The distribution of all T/R values is related to the distribution of T given R by the equality $P(T/R|R) = P(T|R)$. Therefore, an upper limit for the variance of each T/R value was obtained by assuming the hypergeometric case of $K = 1$. A conservative estimate of the error mean square was calculated by averaging the above variances. The maximum and minimum variances differed in a ratio of 3 to 1, well within the limits of robustness for analysis of variance (ANOVA) techniques (Anderson and McLean 1974). Since an excellent normal approximation to the distribution of T/R is available for $K = 1$, it is assumed that the assumption of normality is sufficiently accurate in this study to permit the use of ANOVA techniques.

A more informative statistical analysis was designed to test H_0: $K = 1$ (Cd exposure has no effect on prey vulnerability) versus H_1: $K > 1$ (Cd exposure increases prey vulnerability) for each treatment. To pool the information of three separate runs per Cd treatment, the value $\sum_{exp} = \sum_{i=1}^{3} P(T \geq T_i, \exp|R_iK = 1)$ was chosen as the test statistic. A computer program was devised to calculate distributions of $\sum$, where $\sum$ is the random variable corresponding to $\sum_{exp}$ for different K and (R_1, R_2, R_3) values. H_0 was accepted if $P(\sum \leq \sum_{exp}|K = 1), (R_1, R_2, R_3) > 0.05$ and was rejected if $P(\sum \leq \sum_{exp}|K = 1), (R_1, R_2, R_3) \leq 0.05$.

Confidence intervals for K were obtained through a method related to the hypothesis test. The criteria for confidence limits (K_L, K_U) are the following:

$$K_L = \max K|K: P(\sum \leq \sum_{exp}|K) \leq \alpha/2\},$$

$$K_U = \min K|K; P(\sum' \leq \sum'_{exp}|K) \leq \alpha/2\}.$$

For K'_U, $\sum_{exp} = \sum_{i=1}^{3} P(T \leq T_{exp}|R_i, K = 1)$ and $\sum'$ is the random variable corresponding to $\sum'_{exp}$.

The maximum likelihood estimator (MLE) for the K value of a given test was obtained by looking for the value of K that maximizes $\pi_{i=1}^{3} P(T = T_{exp}K, R_i)$.

This statistical procedure has several advantages over the use of the chi-square statistic typically used to analyze data from predator–prey studies. The use of the MLE for K not only shows when predation pressure is significantly different on selected groups of prey but yields quantitative information as well. When K is significantly different from 1.0, it is useful to discern the relative increase or decrease in prey selection by the predator, as measured by how far the MLE is from 1.0. A detailed description of the statistical methods used in this study may be obtained by requesting a copy of no. 490 Mimeograph Series: "A more informative statistical analysis for predator–prey studies" from the Statistics Department, Purdue University, West Lafayette, Indiana 47907.

Results and Discussion

A two-way analysis of variance showed a significant difference ($P < 0.001$) between the eight Cd treatments but not between the three experimental tanks. There was no significant tank-treatment interaction.

A Newman–Keuls sequential range test showed that the proportion of treated fish eaten was significantly greater following acute exposure to 0.375 and 0.500 mg Cd/L than for the lesser concentrations. While the data indicate that a greater proportion of treated minnows were eaten after a 21-d exposure to 0.050 mg Cd/L than after 0.019 or 0.013 mg Cd/L treatments, the only significant difference detected by the Newman–Keuls test was between those fish exposed to Cd levels of 0.025 and 0.013 mg Cd/L.

Experimental results, MLE's, confidence limits, and

TABLE 3. Experimental results (T_{exp}), maximum likelihood estimators (MLE), 90% confidence intervals, and hypotheses test for *P* values for 48-h exposure tests.

mg Cd/L	Tank no.	*R*	T_{exp}	MLE (for *K*)	90% CI (*K*)	$P(\sum \leq \sum_{exp} \mid K = 1)$
0.500	1	18	12	2.55	(1.8, 6.7)	0.000
	2	16	11			
	3	20	13			
0.375	1	20	13	3.33	(2.1, 6.9)	0.000
	2	21	15			
	3	19	14			
0.250	1	16	9	0.77	(0.4, 1.3)	0.820
	2	19	8			
	3	16	6			
0.050	1	16	8	0.49	(0.4, 0.8)	0.937
	2	12	3			
	3	18	6			

TABLE 4. Experimental results (T_{exp}), maximum likelihood estimators (MLE), 90% confidence intervals, and hypotheses test for *P* values for 21-d exposure tests.

mg Cd/L	Tank no.	*R*	T_{exp}	MLE (for *K*)	90% CI (*K*)	$P(\sum \leq \sum_{exp} \mid K = 1)$
0.050	1	24	14	1.84	(1.3, 4.0)	0.004
	2	20	12			
	3	21	13			
0.025	1	21	15	3.95	(1.7, 5.2)	0.000
	2	20	12			
	3	20	17			
0.019	1	21	11	1.36	(0.8, 1.9)	0.214
	2	24	11			
	3	20	14			
0.013	1	20	8	0.89	(0.5, 1.4)	0.614
	2	17	11			
	3	21	9			

hypothesis test *P* values are listed in Tables 3 and 4. This study indicates that exposure to Cd for 48 h significantly increased ($P < 0.005$) the vulnerability of fathead minnows to predation by largemouth bass at concentrations of 0.50 and 0.375 mg Cd/L but not at 0.25 or 0.05 mg Cd/L. A two-sided test of $H_0: K = 1$ indicated that acute exposure of the minnows to 0.05 mg Cd/L actually decreased prey vulnerability.

While acute exposure of the fathead minnows to 0.05 mg Cd/L appears to have decreased prey vulnerability, 21-d exposure to 0.05 mg Cd/L had an opposite effect. The probability of treated fish being eaten was significantly greater following 21-d exposure to 0.05 and 0.025 mg Cd/L than the probability of controls being selected by the largemouth bass. No effect on prey vulnerability was detected following exposure to 0.019 or 0.013 mg Cd/L.

The decrease in prey vulnerability observed in fish acutely exposed to 0.05 mg Cd/L may be an example of "sufficient challenge," defined by Smyth (1967) as an apparent benefit to organisms from small doses when large doses will cause injury. Several other examples of sufficient challenge to fish are found in the literature (Jackson et al. 1970; Pickering and Gast 1972; Brown 1976).

The results of these tests indicate that the predator–prey interactions of largemouth bass and fathead minnows are very sensitive to low levels of Cd. Pickering and Gast (1972) determined the acute and chronic toxicity of Cd to fathead minnows and reported a maximum allowable toxicant concentration (MATC) of 57–37 μg Cd/L in hard water. At chronic Cd concentrations above 57 μg Cd/L, a decrease in embryo survival was noted. Therefore, prey vulnerability in this study was increased after the 21-d exposure to Cd levels below the MATC reported in the literature.

During the actual predator–prey trials, we attempted to determine what alterations in behavior may have contributed to the increased vulnerability of the treated prey. We found, however, that our recognition of individuals within a school was hindered by the angle from which we observed. To adequately differentiate between treated

and control fish within a school, it was necessary to incorporate the use of glass-windowed observation tanks into the study. Several interesting differences in the behavior of fish exposed to 0.45 mg Cd/L for 48 h were observed. Many of the behavioral differences were quite subtle and required prior familiarity with the normal behavior of the fish in order to be readily detectable. While both treated and control fish schooled, cadmium-treated members often changed direction within the school, orienting perpendicular to the axis of the school for several seconds before returning to their original position. The literature suggests that the primary survival value of schooling behavior is as an antipredator device (Cushing and Harden Jones 1968; Pitcher 1973; Taylor 1976); therefore, anything that interferes with schooling potentially increases prey vulnerability. Since the success of the school as an antipredator device depends on the interresponses from each fish, altered behavior of an individual as a response to a toxicant not only increases the vulnerability of that member to predation but the entire school as well.

When first placed in the observation tanks, treated fathead minnows were more aggressive than controls, repeatedly nipping other fish on the caudal fin. This difference subsided within 30 h after treatment. While more aggressive when initiating an encounter with other members of the population, the aggressive behavior was not consistent, since treated fish were displaced most frequently by the tail nipping, a phenomena that persisted after the decrease in aggressive behavior.

The significance of these behavioral alterations remains speculative; however, it does appear that they merit further consideration. As Warner et al. (1966) have pointed out, one type of behavioral change is not necessarily a more valuable indicator than another if it is assumed that deviation from the norm of any process as a result of toxicant exposure is likely to be deleterious. That behavioral alterations did occur below Cd levels considered safe for fathead minnows (Pickering and Gast 1972) is certainly of concern and suggests that behavioral studies should be used in conjunction with bioassays in determining water quality criteria.

If behavioral studies are to be of value, great care must be taken to make certain that results are indicative of occurrences in natural systems. Altered predator–prey behavior is important to the individual, the population, and the ecosystem. The methods used in this study appeared to be satisfactory; however, addition of an observation window in the side of the tank is recommended. The time required for the predator to ingest 50% of the prey ranged from 3 to 7 d, with a mean of 5 d. We felt that this allowed time for the predator to detect any possible changes in prey-fish behavior elicited by exposure to the Cd. Both the predator and the prey fish utilized the artificial vegetation to seek cover, with the predator often inhabiting the weed cluster. Bams (1967) was of the opinion that an environment that provides adequate space and cover allows for factors such as behavioral alterations rather than factors primarily related to swimming performance to determine differences in prey vulnerability between two groups. We found that the minnows schooled more often and more tightly in the round experimental tanks than in the rectangular observation tanks.

This study has shown that behavioral changes induced by sublethal Cd exposure increase the vulnerability of fathead minnows to predation by largemouth bass. Predator–prey interactions appear to be sensitive indicators of toxicity and have the additional advantage of being readily discernible in a short amount of time. This study suggests that qualitative changes in behavior, detected by observation, merit reporting in conjunction with measured quantitative changes to provide a more complete picture of behavioral toxicology.

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Effect of Double Freezing and Subsequent Long-Term Refrozen Storage at –23°C on the Quality of Inshore Male Capelin (*Mallotus villosus*)

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Botta, J. R., and D. H. Shaw. 1978. Effect of double freezing and subsequent long-term refrozen storage at −23°C on the quality of inshore male capelin (*Mallotus villosus*). J. Fish. Res. Board Can. 35: 452–456.

Whole inshore male capelin (*Mallotus villosus*) were stored at −23°C for 2 mo (C2), or 6 mo (C6) prior to thawing, beheading and eviscerating, and refreezing. Though the quality of the twice-frozen product was in both cases inferior to a once-frozen sample, it was still quite acceptable after 2 yr of refrozen storage. As expected, quality was superior in the C2 samples, but in both sets of samples taste deteriorated to a greater extent than texture. Chemical measurement of peroxide value indicated a possible development of rancidity that could not be detected by sensory analysis. Considerable lipid hydrolysis occurred, with the free fatty acids (FFA) at least doubling during storage; increases were greater in C6. In both experiments FFA production correlated with texture, taste, and with extractable protein nitrogen (EPN). Dimethylamine (DMA), trimethylamine (TMA), hypoxanthine, and EPN appeared to be good indicators of storage time and sensory quality.

Key words: capelin, dimethylamine (DMA), extractable protein nitrogen (EPN), free fatty acids (FFA), hypoxanthine, peroxide value, refrozen storage, taste, texture, trimethylamine

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Des capelans (*Mallotus villosus*) mâles entiers capturés près de la côte ont été entreposés à −23°C durant 2 mo (C2) ou 6 mo (C6) pour ensuite être décongelés, étêtés et éviscérés, et recongelés. Bien que la qualité du produit congelé deux fois soit dans les deux cas inférieure à celle de l'échantillon congelé une fois, le produit est encore assez acceptable après 2 ans d'entreposage une fois recongelé. Comme on peut s'y attendre, la qualité est supérieure dans les échantillons C2. Dans les deux séries d'échantillons, toutefois, il y a détérioration de la saveur à un degré plus grand que la texture. Le dosage chimique de l'indice de peroxyde indique le développement possible d'une rancidité qui n'a pu être détectée par l'analyse sensorielle. Il se produit une hydrolyse considérable des lipides, la teneur en acides gras libres faisant plus que doubler durant l'entreposage; les augmentations sont plus grandes dans les C6. Dans les deux expériences, la production d'acides gras libres est liée à la texture, à la saveur et à la teneur en azote des protéines extractibles. La diméthylamine (DMA), la triméthylamine (TMA), l'hypoxanthine et l'azote des protéines extractibles semblent être de bons indicateurs de la durée d'entreposage et de la qualité sensorielle.

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Capelin (*Mallotus villosus*) spawn on Newfoundland beaches in June and July, and are used domestically but not commercially. During this period, fish processing plants, busy processing the more valuable trap-caught cod, do not generally have the capacity to process inshore capelin simultaneously. We have previously discussed the long-term storage of once-frozen, ungutted, inshore male capelin (Shaw and Botta 1977). Commercial practice, however, is more likely to freeze uneviscerated capelin in blocks for relatively short periods (2–3 mo) before thawing, gutting, beheading, repacking, refreezing, and storing. The work reported in this paper simulates the above practice.

Double freezing has previously been used successfully with a number of species (Burgess et al. 1965; Connell 1975; Hean and Karsti 1965; and MacCallum et al. 1966, 1967, 1968, 1969).

Materials and Methods

Inshore male capelin were caught and frozen (15 h after catching, 12 h in ice) as previously described (Shaw and Botta 1977). Frozen blocks of capelin were stored at −23°C for 2

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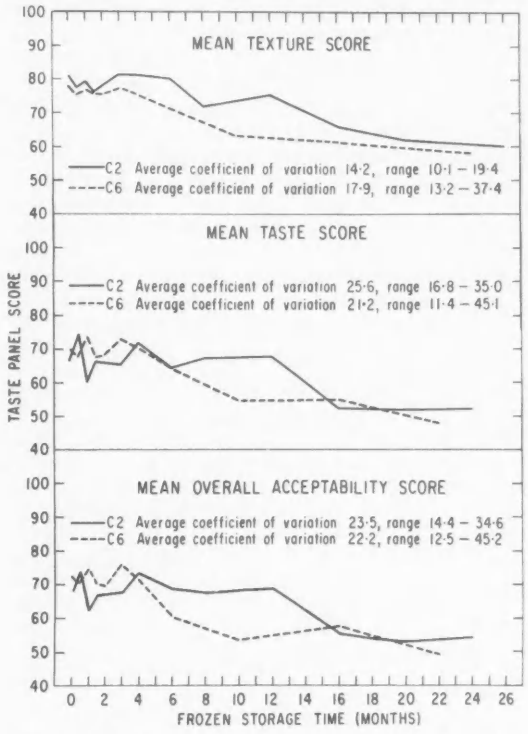
MEAN TEXTURE SCORE
C2 Average coefficient of variation 14·2, range 10·1 – 19·4
C6 Average coefficient of variation 17·9, range 13·2 – 37·4
MEAN TASTE SCORE
C2 Average coefficient of variation 25·6, range 16·8 – 35·0
C6 Average coefficient of variation 21·2, range 11·4 – 45·1
MEAN OVERALL ACCEPTABILITY SCORE
C2 Average coefficient of variation 23·5, range 14·4 – 34·6
C6 Average coefficient of variation 22·2, range 12·5 – 45·2
TASTE PANEL SCORE
FROZEN STORAGE TIME (MONTHS)

FIG. 1. Sensory analysis of refrozen inshore male capelin stored at −23°C.

and 6 mo, experiments C2 and C6, respectively. When the respective storage times had elapsed, the blocks were thawed in running tap water, gutted, beheaded, packed in 454-g capacity waxed cardboard boxes, and frozen in a horizontal plate freezer. Each box contained 12–15 male capelin. Refrozen capelin from C2 and C6 were stored at −23°C for up to 24 and 22 mo, respectively. At various times samples consisting of three boxes of capelin for chemical analyses and three boxes of capelin for sensory analyses were removed and stored at −40°C until analyzed.

Texture, taste, and overall acceptability were measured by an analytical panel of five judges using a 100-point (10, 20 . . . 80, 100) scale (descriptive for texture and taste and hedonic for overall acceptability) that was defined such that a score of 40 was given to a sample judged borderline with respect to the parameter being sampled (Shaw and Botta 1975). Each judge evaluated four separate samples (fish) for each storage time giving a total of 20 observations for texture, taste, and overall acceptability per storage time.

Free fatty acid (FFA), hypoxanthine, dimethylamine (DMA), trimethylamine (TMA), peroxide, and extractable protein nitrogen (EPN) values were all quantitatively determined by methods described or annotated by Shaw and Botta (1977).

Correlation coefficients were determined using the storage time means of the variables, noting that correlation indicates an association only and not necessarily a cause-and-effect relationship.

For the illustrations, the average and range of the coefficients of variation refer to storage time means.

Results and Discussion

Sensory Analysis

Texture — After approximately 2 yr of refrozen storage the texture of inshore male capelin from both experiments was quite acceptable (Fig. 1). The mean texture scores of C2 and C6 capelin immediately after refreezing were substantially lower than the mean texture score of 92.0 for capelin frozen shortly after catching (Shaw and Botta 1977). (Unless otherwise indicated any comparisons to once-frozen inshore capelin refer to this paper.) The initial time of frozen storage may be important because the mean texture scores of experiment C6 started to decrease several months before those of experiment C2 (Fig. 1).

After 24 mo of refrozen storage the mean texture score of C2 capelin was only 8 points lower than that of capelin that had been frozen, shortly after catching, and stored at −23°C for 24 mo without double freezing. Similarly, the mean texture score of experiment C6 was 10.5 points lower than that of capelin frozen once and stored at −23°C for 24 mo. These results indicated that freezing, thawing, processing, and refreezing only moderately affected the texture of frozen stored capelin when compared to single freezing.

Taste — During approximately 2 yr of refrozen storage, the taste scores of inshore male capelin substantially decreased but were still quite acceptable to the end (Fig. 1). As with texture scores, taste scores of experiment C6 started to decrease several months before those of experiment C2. The mean taste scores at 0 refrozen storage time for both experiments were substantially lower than the mean taste score of 87.25 for capelin frozen shortly after catching. This was presumably due to deterioration during the different initial periods of frozen storage and to the effect of refreezing.

After several months of frozen storage and approximately 2 yr of refrozen storage, the final mean taste scores of C2 and C6 capelin were essentially the same as the final mean taste score of inshore male capelin frozen shortly after catching and stored at −23°C for 24 mo, indicating that double freezing, when compared to single, had little effect on taste.

Overall acceptability — Capelin frozen shortly after catching had a mean overall acceptability score of 89.8. During the initial freezing, frozen storage, thawing, processing, and refreezing, the mean overall acceptability scores decreased approximately 20 points for C2 and C6 capelin, indicating that collectively the above processes had a substantial effect (Fig. 1). During the subsequent 24 and 22 mo of refrozen storage, the mean overall acceptability scores decreased a further 14 and 22 points, respectively, indicating that although double freezing followed by approximately 2 yr of refrozen storage substantially affected overall acceptability, the fish were still edible (Fig. 1).

The mean overall acceptability scores of C2 capelin were moderately higher than those of C6 capelin, possibly

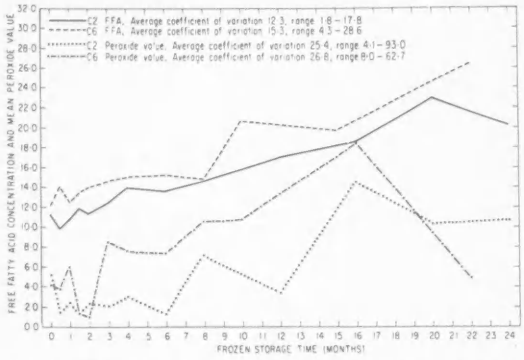
C2 FFA. Average coefficient of variation 12.3, range 1.6 – 17.8
C6 FFA. Average coefficient of variation 15.3, range 4.3 – 28.6
C2 Peroxide value. Average coefficient of variation 25.4, range 4.1 – 93.0
C6 Peroxide value. Average coefficient of variation 26.8, range 8.0 – 62.7
FREE FATTY ACID CONCENTRATION AND MEAN PEROXIDE VALUE
FROZEN STORAGE TIME (MONTHS)

FIG. 2. Changes in FFA (percent of total lipid) and peroxide value (meq $Na_2S_2O_2$/100 g fat) of refrozen inshore male capelin stored at −23°C.

because of the extra 4 mo of storage prior to processing of the latter samples.

In general, the texture, taste, and overall acceptability data of both experiments indicated that inshore male capelin kept better than would have been expected during approximately 2 yr of refrozen storage preceded by initial periods of up to 6 mo of frozen storage of the whole fish. Changes due to double freezing and subsequent refrozen storage of capelin are less severe than those for Newfoundland trap-caught cod stored at −23°C (Dyer et al. 1962), refrozen trawl-caught cod stored at −18°C (Peters et al. 1968), and refrozen trap-caught and trawl-caught cod stored at −23°C (MacCallum et al. 1966). As inshore capelin contain approximately 10 times the fat content of Atlantic cod, these results tend to substantiate Heen and Karsti's (1954) observations that fatty fish withstand refreezing better than lean fish.

CHEMICAL ANALYSES

Changes associated with lipid — Peroxide value increased to a maximum at 16 mo of refrozen storage in both experiments and then decreased thereafter (Fig. 2). This pattern has been observed with redfish fillets stored at −23°C (Dyer et al. 1956) and with raw herring fillets stored at −15°C (Bosund and Ganrot 1970). It was questionable if these substantial increases in mean peroxide values indicated any appreciable development of rancidity. The rancidity (if any) was probably at subthreshold levels as it was not detected by the taste panel. Thus, inshore male capelin that contain approximately 3% lipid with an iodine number of approximately 130 kept better than would have been predicted. The peroxide values in C2 and C6 capelin increased to slightly and moderately higher values, respectively, than the peroxide values of capelin frozen shortly after catching and stored at −23°C for 24 mo. The differences in the frozen storage times prior to processing and refreezing between C2 and C6 capelin explained why the peroxide values of C6 capelin were generally greater than those of C2 (Fig. 2). For C2 capelin peroxide value significantly ($P \leq 0.05$) correlated with texture, taste, and overall acceptability (Table 2). However, this correlation did not hold for C6 capelin.

There was a consistently greater FFA increase with refrozen storage time for C6 capelin samples when compared to those of C2 (Fig. 2). This was at least partially due to the longer frozen storage time prior to thawing of the C6 capelin samples. The above results indicated that considerable lipid hydrolysis occurred in both experiments (Fig. 2). In general, the above changes in FFA concentration were slightly smaller than those of trawled redfish, dressed, frozen in rigor, stored for 9 wk at −23°C, then thawed in water, refrozen, and stored at −23°C for 81 wk (MacCallum et al. 1967). Changes in FFA concentration of the present experiments were substantially less than those of once-frozen lake whitefish (*Coregonus clupeaformis*) muscle stored at −10°C, which contained slightly more lipid (3.48% at 0 time) than the capelin used in the present experiments (Awad et al. 1969). The change of FFA concentration in the present experiments followed a pattern similar to that of once-frozen Baltic herring (Bosund and Ganrot 1969) but was slightly different from the pattern observed in inshore male capelin frozen shortly after catching and stored at −23°C for 24 mo. Free fatty acid concentration of both experiments significantly ($P \leq 0.05$) correlated with texture, taste, and overall acceptability (Table 2). It is noteworthy that FFA concentration changes significantly ($P \leq 0.05$) correlated with extractable protein nitrogen (EPN) changes (Table 3).

Miscellaneous chemical changes — There was a moderate increase in both DMA and TMA for experiments C2 and C6 (Table 1). The increase in DMA for both experiments appeared to be considerably smaller than that observed with once-frozen Pacific hake (*Merluccius productus*), silver hake (*Merluccius bilinearis*), pollock (*Pollachius virens*), and cusk (*Brosme brosme*) (Babbitt et al. 1972; Castell et al. 1971) and substantially greater than that observed with once-frozen Atlantic halibut (*Hippoglossus hippoglossus*), American plaice (*Hippoglossoides platessoides*), redfish (*Sebastes marinus*), and Atlantic wolffish (*Anarhichas lupus*) (Castell et al. 1971). Changes in DMA concentration significantly ($P \leq 0.05$) correlated with texture, taste, and overall acceptability for both experiments (Table 2) indicating that DMA may be a useful indicator of quality. Changes in DMA concentration significantly correlated with changes in EPN and FFA for both experiments (Table 3). This observation is similar to that of Castell who observed that with once-frozen cod the DMA increase appeared to correspond to the increase in FFA and the decrease in protein solubility (Castell et al. 1968, 1970). The changes in DMA concentration in C2 capelin were quite similar to that observed with once-frozen inshore male capelin. The DMA values of C6 capelin were initially lower than expected but by the end of the 22 mo of refrozen storage were slightly greater than those of the once-frozen inshore male capelin. Thus, in the present experiments, the effect of refrozen storage on DMA concentration depended on the length of frozen storage prior to thawing.

TMA concentration of C2 capelin increased to a moder-

TABLE 1. Changes in DMA (mg DMA-N/100 g fish), TMA (mg TMA-N/100 g fish), hypoxanthine (mg/100 g fish), and EPN (g/100 g fish) of inshore male capelin frozen shortly after catching, stored at −23°C for several months, then thawed, processed, refrozen, and stored at −23°C. Each value is the average of three observations.

Storage time (mo)	DMA		TMA		Hypoxanthine		EPN	
	C2	C6	C2	C6	C2	C6	C2	C6
0	0.237	0.214	0.488	0.428	17.3	14.6	1.42	1.26
0.5	0.159	0.275	0.784	0.689	17.5	20.9	1.34	1.34
1	0.178	0.251	0.711	0.868	19.0	14.9	1.42	1.05
1.5	0.198	0.257	0.435	0.409	20.4	15.2	1.48	1.19
2	0.184	0.245	0.421	0.613	18.9	19.3	1.27	1.34
3	0.239	0.269	0.613	0.664	16.9	17.9	1.52	1.17
4	0.334	0.236	0.358	0.655	14.5	16.8	1.22	1.13
6	0.263	0.362	0.268	0.779	18.6	20.4	1.28	0.88
8	0.380	0.432	0.689	0.907	15.4	29.0	0.68	0.72
10		0.409		0.923		25.3		0.70
12	0.382		0.638		17.3		0.81	
16	0.489	0.705	0.790	1.002	24.9	24.7	0.43	0.32
20	0.458		0.751		26.3		0.48	
22		0.864		0.955		30.8		0.30
24	0.633		1.011		27.8		0.49	

TABLE 2. Correlation coefficients (r) of chemical parameters vs. sensory parameters for C2 and C6 capelin.* Significant at the 5% level ($P \leq 0.05$); **significant at the 1% level ($P \leq 0.01$).

Experiment	Chemical parameter	Correlation coefficients (r)			n
		Texture	Taste	Overall acceptability	
C2	Peroxide value	−0.8580**	−0.7923**	−0.8166**	12
C6	Peroxide value	−0.5374	−0.4020	−0.4149	11
C2	FFA	−0.8687**	−0.7576*	−0.7915**	12
C6	FFA	−0.9526*	−0.9073**	−0.9051**	11
C2	DMA	−0.8573**	−0.6959*	−0.7166**	12
C6	DMA	−0.9332**	−0.8814**	−0.8455**	11
C2	TMA	−0.7394**	−0.5532	−0.6232*	12
C6	TMA	−0.7811**	−0.6558*	−0.6896*	11
C2	Hypoxanthine	−0.8742**	−0.9041**	−0.9221**	12
C6	Hypoxanthine	−0.9343**	−0.9164**	−0.9119**	11
C2	EPN	+0.8934**	+0.6652*	+0.7148**	12
C6	EPN	+0.9423**	+0.8718**	+0.8637**	11

TABLE 3. Correlation coefficients (r) between various chemical parameters of C2 and C6 capelin. *Significant at the 5% level ($P \leq 0.05$); **significant at the 1% level ($P \leq 0.01$).

Experiments	Chemical parameter	Correlation coefficients (r)			n
		DMA	TMA	EPN	
C2	FFA	0.9027**	0.4897	−0.8923**	13
C6	FFA	0.9079**	0.6586*	−0.8415**	12
C2	DMA			−0.9065**	13
C6	DMA			−0.9319**	12
C2	TMA			−0.6142*	13
C6	TMA			−0.8159**	12

ately greater level than that observed with DMA (Table 1) whereas with C6 capelin TMA increased to a level only slightly greater than DMA (Table 1). With both experiments the peak TMA concentration was very similar to that observed with once-frozen inshore male capelin, indicating that double freezing and refrozen storage did not substantially affect TMA concentration. Since the method of Tozawa et al. (1971) was used to measure TMA the increase in concentration was probably real and not an artifact, but rather either the product of very slow bacterial degradation or the action of the reducing enzyme found in the red muscle of pelagic fish (Castell 1970). The above results are in marked contrast to those observed with frozen cod (Castell et al. 1970). Trimethylamine changes significantly ($P \leq 0.05$) correlated with texture and overall acceptability for C2 capelin and correlated with texture, taste, and overall acceptability for C6 capelin, indicating that TMA may be a useful quality indicator for refrozen inshore male capelin (Table 2). Changes in TMA concentration also significantly correlated with changes in EPN for both experiments and with changes in FFA concentration for C6 capelin (Table 3). However, these significant correlations may be fortuitous with no real importance.

Hypoxanthine concentrations at 0 refrozen storage time were moderately and slightly greater for experiments C2 and C6, respectively, than the value for inshore male capelin frozen shortly after catching and stored at −23°C for 0.5 mo (Table 1). This may be at least partially due to the frozen storage time prior to thawing. The hypoxanthine concentrations were somewhat erratic during the first 12 mo for C2 capelin and during the first 4 mo for C6 capelin. In general, the pattern of hypoxanthine change was similar to that observed with inshore capelin frozen shortly after catching and stored at −23°C for 24 mo. The magnitude of change observed with the present experiments was substantially greater than that observed in once-frozen cod stored at −20°C (Jones 1963). Hypoxanthine concentration changes significantly correlated with texture, taste, and overall acceptability for both experiments (Table 2).

Extractable protein nitrogen (EPN) value decreased substantially during approximately 2 yr of refrozen storage (Table 1). The rate of decrease of EPN was slightly slower for C2 capelin and slightly faster for C6 capelin than that of refrozen cod stored at −18°C (plate frozen and analyzed prior to thawing in water) (Peters et al. 1968). Results of the present experiments are similar

to that observed in once-frozen inshore male capelin stored at −23°C for 24 mo indicating that double freezing and refrozen storage did not substantially affect EPN values. Extractable protein nitrogen significantly ($P \leq 0.05$) correlated with texture, taste, and overall acceptability of both experiments, suggesting that EPN may be a useful index of quality for refrozen inshore male capelin (Table 2). Free fatty acids, DMA, and TMA significantly ($P \leq 0.05$) correlated with EPN changes for both experiments (Table 3). Since formaldehyde is produced on an equimolar basis with DMA, it is probably the effect of formaldehyde on protein insolubility (Castell 1971; Castell et al. 1973) that accounts for the significant correlation between DMA and EPN. Although FFA significantly correlated with EPN, it may not indicate any direct relationship because FFA correlated with DMA and DMA correlated with EPN (Table 3). A similar observation has been noted with gadoid species (Castell 1971).

The chemical data indicated that values for TMA and EPN of the present experiments appeared similar to those of once-frozen inshore male capelin stored at −23°C, whereas the values for peroxide value, FFA, DMA, and hypoxanthine of the present experiments appeared to be slightly or moderately different from those of once-frozen inshore male capelin stored at −23°C for 24 mo.

The present experiments indicated that FFA, DMA, TMA, hypoxanthine, and EPN may be useful indicators of quality for refrozen inshore male capelin.

In conclusion, the chemical and sensory results of the present experiments indicated that double freezing appeared to be a practical and useful means of preserving inshore male capelin (*Mallotus villosus*), obviating the difficulties caused by plants being unable to fully process the capelin immediately after harvest.

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Storage Stability of Channel Catfish (*Ictalurus punctatus*) in Relation to Dietary Level of α-Tocopherol[1]

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O'KEEFE, T. M., AND R. L. NOBLE. 1978. Storage stability of channel catfish (*Ictalurus punctatus*) in relation to dietary level of α-tocopherol. J. Fish. Res. Board Can. 35: 457–460.

Fingerling channel catfish (*Ictalurus punctatus*) 12–15 cm long were fed semipurified diets containing 0, 5, 10, 20, 40, and 80 mg of *dl*-α-tocopherol acetate per 100 g dry diet, and slaughtered after 97 d. Storage stability of fillets for the different treatments was determined by 2-thiobarbituric acid analysis after 3 mo frozen storage and after 3 mo freezing followed by refrigeration for 3 d. Fillets from fish fed the two lowest α-tocopherol levels exhibited significant oxidative rancidity after frozen storage. All higher levels showed similar but negligible oxidation. The 3 d of additional refrigerated storage significantly increased the amount of fat oxidation in all fillets from fish fed less than 40 mg/100 g. These results strongly indicate that increased dietary levels of α-tocopherol are effective in increasing the storage stability of catfish fillets.

Key words: storage stability, channel catfish, antioxidants, *dl*-α-tocopherol, 2-thiobarbituric acid

O'KEEFE, T. M., AND R. L. NOBLE. 1978. Storage stability of channel catfish (*Ictalurus punctatus*) in relation to dietary level of α-tocopherol. J. Fish. Res. Board Can. 35: 457–460.

Des fingerlings de barbue de rivière de longueurs (*Ictalurus punctatus*) comprises entre 12 et 15 cm ont été nourris à des régimes semi-purifiés contenant 0, 5, 10, 20, 40 et 80 mg d'acétate de dl-α-tocophérol par 100 g de nourriture sèche et ont été tués après 97 jours. Nous avons déterminé par analyse de l'acide 2-thiobarbiturique la stabilité à l'entreposage des filets pour les différents traitements, après 3 mo d'entreposage congelé et après 3 mo d'entreposage congelé suivis de 3 jours d'entreposage refroidi. Les filets de poissons ayant reçu les deux niveaux les plus bas d'α-tocophérol montrent une rancidité par oxydation significative après entreposage congelé. Tous les niveaux plus élevés montrent une oxydation identique mais négligeable. Les 3 jours d'entreposage refroidi supplémentaire augmentent de façon significative la quantité d'oxydation des graisses dans tous les filets des poissons ayant reçu moins de 40 mg/100 g. Ces résultats indiquent assez clairement q'une augmentation des niveaux alimentaires de l'α-tocophérol accroissent la stabilité à l'entreposage des filets de barbue de rivière.

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SUCCESSFUL marketing of many seafood products is contingent on long-term frozen storage. During such storage, oxidative rancidity of fish tissue lipids occurs, leading to product degradation. Chemical antioxidants, when applied directly on processed fishery products prior to frozen storage, are effective as deterrents to this type of spoilage (J. Licciardello et al. unpublished data). However, no attempts have been made to increase the storage stability of these highly perishable products through dietary administration of natural antioxidants. Recent studies involving rancidity of pork and poultry fats have shown that increased dietary levels of the natural antioxidant α-tocopherol improve quality and flavor of the flesh (Astrup 1973; Laksesvela 1960). Our study evaluates this nutritional approach as a means of controlling fat rancidity in frozen channel catfish (*Ictalurus punctatus*) fillets.

Methods

Fifteen fingerling channel catfish having an average weight of 26.3 g were stocked in each of 24 aquaria for feeding experiments, which allowed four replications of six tocopherol levels. Each aquarium had a capacity of 57 L and was supplied with heated well water at a rate of 7.5 L/h. Water temperatures during the study period were maintained between 22 and 26°C. Periodic measurements of dissolved oxygen ranged from 6.0 to 7.0 mg/L.

A basal tocopherol-free diet (Table 1), similar to the 30% protein diet of Krisnandhi and Shell (1965), was used as one experimental level and was supplemented with 5, 10, 20, 40, and 80 mg of *dl*-α-tocopherol acetate per 100 g of dry diet. A vitamin supplement (Halver and Coates 1957) supplied all other essential vitamins. Tocopherol-stripped corn oil was used as the source of fat.

Experimental diets were prepared by mixing the dry ingredients and the oil in a 5% agar solution. A meat grinder was used to form the feed into strings approximately 4 mm diam, which were repeatedly forced through the grinder until

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TABLE 1. Channel catfish test diet.

Complete test diet (g)		Vitamin mix (mg)		Mineral mix (g)	
Vitamin-free casein (68% protein)	45.0	Thiamin	5.00	Calcium biphosphate	13.580
Dextrin	20.00	Riboflavin	20.00	Calcium lactate	32.700
Tocopherol stripped corn oil	10.0	Pyridoxine	5.00	Ferric citrate	2.970
Mineral mix (as shown)	4.0	Choline	500.00	Magnesium sulfate	13.200
Vitamin mix (as shown)	1.0	Nicotinic acid	75.00	Potassium phosphate	23.980
Agar	5.0	Ca-panthothenate	50.00	Sodium biphosphate	8.720
α-cellulose	15.0	Inositol	200.00	Sodium chloride	4.350
Water	100.0	Biotin	0.50	$AlCl_3\ 6H_2O$	0.015
Total diet as fed	200.0	Folic acid	1.50	KCl	0.015
		L-ascorbic acid	100.00	CuCl	0.010
		B_{12} mix (0.5 mL)[a]	0.01	$MnSO_4\ H_2O$	0.080
		Menadione (K)	4.00	$CoCl_2\ 6H_2O$	0.100
		D_3	0.50	$ZnSO_4\ 7H_2O$	0.300
		A	1.10		
		dl-α-tocopherol acetate[b]	—		

[a]Vitamin B_{12} (10 mg/500 mL water). [b]Six levels — 0, 5, 10, 20, 40, 80.

the agar cooled enough to form small pellets when extruded. Diets were freshly prepared every 7 d and stored in sealed plastic containers at 4°C.

Fish in each aquarium were fed the prepared diet at a rate of 3% of their body weight per day with adjustments made for mortalities. Every 18–20 d, all aquaria were drained and total weight of fish in each was determined to allow adjustment of feeding rates for growth.

After 97 d all of the fish were removed, weighed, and filleted. Fillets from catfish in each aquarium were divided into two lots, each containing one fillet from each fish, and placed in plastic bags in a freezer at −10°C. Studies of storage stability were conducted by measuring changes in oxidation state of fillets using a modification of the 2-thiobarbituric acid (TBA) method of Tarladgis et al. (1960). This modification consisted of stabilization of fillet homogenates by addition of 2 mL of 5% pyrogallol in water before bringing the final volume up to 110 mL. This sample was then acidified and distilled. An aliquot of the distillate was combined with an equal volume of TBA solution, heated, and absorbance determined on a Beckman B Spectrophotometer at 535 μm.

Due to time commitments, baseline TBA analyses could not be conducted until 15 d after initial storage. At that time one lot of fillets from each aquarium was thawed at room temperature and two replicates of each experimental treatment were combined to reduce the number of samples to be analyzed from 24 to 12. The baseline level of fat oxidation was determined on homogenized samples by the modified TBA technique.

The remaining lot from each aquarium was kept in frozen storage for 3 mo before analysis. At that time each sample of fillets was thawed and subdivided into two equal lots, one for immediate analysis and one for further refrigerated storage. In the first lot, replicates of each dietary level of α-tocopherol were combined to reduce the number of samples to 12 and TBA analyses were conducted. In the remaining lot, replicates were similarly combined and the resultant samples were spread on a wire rack to maximize fat oxidation. These fillets were then held in a refrigerator at 2°C. After 3 d, TBA analyses were performed on the thawed-rerefrigerated samples to determine the extent of fat oxidation.

TBA value (Sinnhuber and Yu 1958), expressed as milligrams of malonaldehyde per kilogram of sample, was used to report the extent of fat rancidity of fillets. These values were calculated by multiplying the optical density (absorbance) of the processed samples by a constant, K. This constant was obtained from standard curves and known dilutions of tetraethoxypropane according to the method of Tarladgis et al. (1960). Their method incorporates variations in weight of sample processed and percent recovery of malonaldehyde. Changes in fat oxidation (ΔTBA) were calculated by subtracting baseline TBA values for replicate samples from the TBA values of samples from the same aquaria after frozen and frozen-refrigerated storage. Analysis of variance and the Student–Newman–Keul's (S–N–K) test (Steel and Torrie 1960) were used to analyze and compare experimental results. Correlation analysis was used to explain the relationship of ΔTBA value to α-tocopherol feeding level. For all statistical analyses in this paper, the 0.05 level of probability is considered significant.

Results and Discussion

Baseline TBA values for the 12 samples, measured after 15 days of frozen storage, ranged from 0.37 to 0.69 for the six tocopherol levels (Table 2). Although these values were low, significant differences among average baseline TBA values were detected. These differences indicated that differential oxidation of samples probably occurred during the short period prior to baseline analysis, since it is unlikely that any differences in oxidation state could have occurred at time of killing.

Differences in the extent of fat oxidation among samples frozen for 3 mo were also found to be significant. Fillets from fish fed the tocopherol-free diet and those fed 5 mg of dl-α-tocopherol acetate per 100 g of dry feed had TBA values greater than 3.0, which were indicative of poor quality (Sinnhuber and Yu 1958). Because of the variation in baseline TBA values, ΔTBA values were calculated for each treatment for the frozen storage period. With the exception of the 80-mg ration, all ΔTBA values were ranked in relation to the amount of dietary α-tocopherol, but ΔTBA values for fillets from fish fed the 10-, 20-, 40-, and 80-mg rations were not

TABLE 2. Mean TBA values and ΔTBA for baseline, frozen, and frozen-refrigerated samples.

Tocopherol level[a]	Baseline TBA[b]	Frozen storage TBA	Frozen storage ΔTBA	Frozen-refrigerated TBA	Frozen-refrigerated ΔTBA
80	0.69	1.73	1.04[e]	1.76	1.06[e]
40	0.37	0.98	0.61[e]	1.22	0.85[e]
20	0.43	1.36	0.93[e]	2.45	2.02[d]
10	0.48	1.54	1.06[e]	2.63	2.15[d]
5	0.59	3.27	2.86[d]	5.03	4.44[e]
0	0.67	6.57	5.90[e]	7.70	7.13[f]

[a]Milligrams of *dl*-α-tocopherol acetate per 100 g of dry feed.
[b]Milligrams of malonaldehyde per kilogram of fillet.
[c,d,e,f]Values within a given column followed by the same superscript are not statistically different ($P < 0.05$).

significantly different from one another (Table 2). Regression analysis indicated that the natural logarithms of ΔTBA values and dietary levels of α-tocopherol up to 40 mg/100 g were linearly related ($r = 0.85$).

Samples frozen for 3 mo, thawed, and refrigerated for 3 d developed levels of fat rancidity that exhibited the same trends in relation to dietary levels of α-tocopherol as those in the frozen storage test (Fig. 1). The correlation coefficient for the regression of natural logarithms of ΔTBA values against dietary α-tocopherol level was highly significant ($r = 0.78$). The ΔTBA values of fillets from the fish other than those fed the 80-mg ration were increased over those for the frozen samples. As found for the frozen samples, an increase occurred in the ΔTBA value corresponding to the 80-mg ration. However, analysis of the treatment means indicated that the extent of fat rancidity was not significantly different between the samples from fish fed the 10- and 20-mg rations or those fed the 40- and 80-mg rations (Table 2).

These data indicate that ΔTBA values, although increased, remain ranked after exposure of fillets to conditions that promoted further oxidative rancidity. Fillets frozen for 3 mo and refrigerated for 3 d were most subject to fat oxidation, as fillets in extended frozen storage might be. Under these conditions, doubling of the dietary α-tocopherol level to 40 mg/100 g of dry diet consistently reduced the extent of change in fillet lipid oxidation (ΔTBA). However, each increase was progressively less effective. The final increase from 40 to 80 mg of *dl*-α-tocopherol acetate per 100 g of dry diet produced no additional protection from oxidative rancidity (Fig. 1). Apparently, for each specific storage condition, the relative efficiency of *dl*-α-tocopherol as an antioxidant in fish decreases with increased dosage. This effect was strongly pronounced in fillets that were frozen for only 3 mo and were therefore less subject to fat oxidation. The ΔTBA values for samples from treatments receiving more than 10 mg/100 g were not significantly different. Therefore, for long-term storage, the selection of higher dietary levels of α-tocopherol to improve the final quality of the fish fillets is important. The most economical levels can be selected by giving consideration to anticipated storage time and the cost-benefit relationship of increased protection.

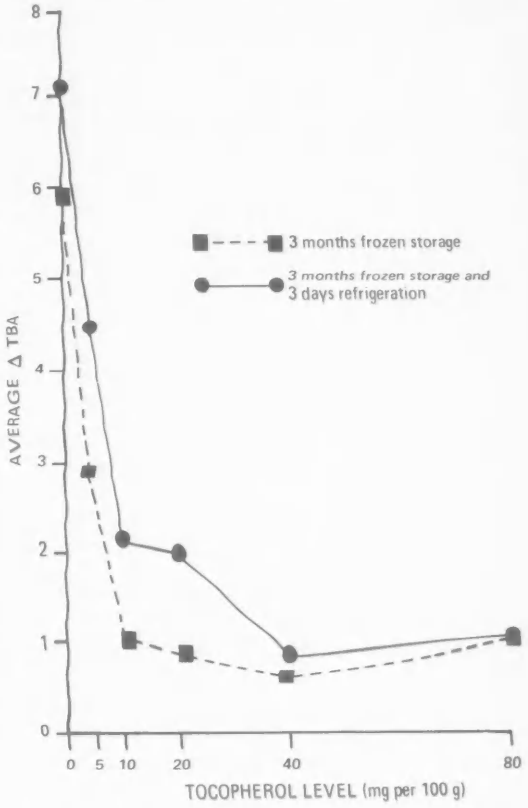

FIG. 1. Average ΔTBA values for frozen and frozen-refrigerated samples.

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Population Dynamics of *Daphnia galeata mendotae* as Modified by Chronic Cadmium Stress

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MARSHALL, J. S. 1978. Population dynamics of *Daphnia galeata mendotae* as modified by chronic cadmium stress. J. Fish. Res. Board Can. 35: 461–469.

Forty populations of *Daphnia galeata mendotae* were maintained for 22 wk (or until they became extinct) in a medium consisting of filtered Lake Michigan water to which *Chlamydomonas reinhardi* and different concentrations of a mixture of labelled (^{109}Cd) plus stable $CdCl_2$ were added 24 h before use. The medium was changed daily Monday through Fridays. Weekly determinations were made of total numbers, average individual dry weight, mean brood size, egg–female ratio, aborted eggs and embryos, and average whole-body ^{109}Cd content. Chronic cadmium stress reduced the populations' average numbers and biomass, while it increased the populations' turnover rates, temporal variability, probability of extinction, and proportion of ovigerous females. For individuals there was increased prenatal mortality, reduced life expectancy, increased average weight, and increased brood size. Due to compensatory increases in the proportion of ovigerous females and mean brood size, the cadmium sensitivity of population attributes was considerably less than that of the most sensitive individual attribute, prenatal mortality. A linear relationship between population carrying capacity (relative mean numbers) and added cadmium concentration indicated that the effective concentration for a 50% reduction of carrying capacity (EC50) is 7.7 μg Cd/L and that for a 1% reduction (EC1) is 0.15 μg Cd/L. An increase of 0.15 μg Cd/L in Lake Michigan itself, barring synergistic interaction with other factors in the lake, would probably not have a detectable effect on the population dynamics of *D. galeata mendotae*.

Key words: zooplankton dynamics, stress ecology, *Daphnia galeata mendotae*, cadmium, heavy metals, toxic substances, Laurentian Great Lakes

MARSHALL, J. S. 1978. Population dynamics of *Daphnia galeata mendotae* as modified by chronic cadmium stress. J. Fish. Res. Board Can. 35: 461–469.

Nous avons maintenu 40 populations de *Daphnia galeata mendotae* durant 22 sem (ou jusqu'à extinction) dans un milieu constitué d'eau filtrée du lac Michigan à laquelle on avait ajouté, 24 h avant emploi, *Chlamydomonas reinhardi* et différentes concentrations d'un mélange de $CdCl_2$ stable et de $CdCl_2$ marqué (^{109}Cd). Nous avons renouvelé quotidiennement le milieu de culture du lundi au vendredi. Nous avons déterminé chaque semaine les nombres totaux d'individus, le poids sec individuel moyen, la taille moyenne de la progéniture, le rapport oeufs:femelles, le nombre d'oeufs et d'embryons avortés, et la teneur moyenne en ^{109}Cd du corps entier. Un stress chronique au cadmium entraîne une diminution des nombres moyens et de la biomasse des populations, et une augmentation des vitesses de renouvellement, de la variabilité dans le temps, de la probabilité d'extinction et de la proportion des femelles oeuvées dans les populations. Pour ce qui est des individus, on constate une mortalité prénatale accrue, une durée de vie moindre, un poids moyen plus grand et une progéniture plus abondante. Par suite d'augmentations compensatoires dans la proportion des femelles oeuvées et de la taille moyenne de la progéniture, la sensibilité des attributs des populations au cadmium est beaucoup moindre que celle de l'attribut individuel le plus sensible, la mortalité prénatale. Une relation linéaire entre la capacité de charge des populations (nombres moyens relatifs) et la teneur en cadmium indique que la concentration effective entraînant une réduction de 50% de la capacité de charge (CE50) est de 7.7 μg de Cd/L et que pour une diminution de 1% (CE1) elle est de 0.15 μg de Cd/L. Une augmentation de 0.15 μg de Cd/L dans le lac Michigan lui-même, hormis une interaction de synergie avec d'autres facteurs dans le lac, n'aurait probablement pas d'effet décelable sur la dynamique des populations de *D. galeata mendotae*.

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CADMIUM has long been recognized as a highly toxic element, but only recently has concern been expressed over the impact of anthropogenic emissions of cadmium into the environment. Man's activities are now contributing more cadmium to freshwater ecosystems than that contributed by natural processes (Fleischer et al. 1974). Crustacean zooplankton appear to be more sensitive to cadmium than either phytoplankton or fish and thus may be critical in determining the overall impact of cadmium

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in plankton-dominated ecosystems such as the Laurentian Great Lakes. Cadmium concentrations <1 μg/L have been reported to be toxic to the freshwater zooplankter *Daphnia magna* (Biesinger and Christensen 1972), whereas cadmium has not been reported to be toxic to phytoplankton at concentrations below 6 μg/L (Klass et al. 1974) or to fish at concentrations below 3.4 μg/L (Benoit et al. 1976). Anderson (1950) reported acute (64 h) cadmium toxicity to *D. magna* at concentrations <1.6 μg/L and stated that the sensitivity of *D. magna* was representative of other zooplankters, including copepods as well as other cladocerans. Studies of cadmium effects on zooplankton have heretofore dealt only with effects on life-history phenomena and attributes of individuals in cohorts (isolated generations). In spite of the recognized importance of zooplankton in lakes, no studies have been made of the chronic (long-term) effects of cadmium on zooplankton populations, and no studies have been reported of cadmium effects on a species of *Daphnia* inhabiting the Great Lakes.

The purpose of this study was to determine the long-term effects of elevated concentrations of cadmium in Lake Michigan water on laboratory populations of *Daphnia galeata mendotae*, a species of some importance in all of the Great Lakes. Emphasis is placed on the effects of chronic cadmium stress on population attributes such as carrying capacity and turnover rates and on analysis of underlying changes in individual attributes and compensatory adjustments to chronic cadmium stress.

Materials and Methods

Daphnia galeata mendotae populations were maintained in 500 mL of medium in 600-mL polypropylene beakers. The medium was renewed daily Monday through Friday and consisted of filtered Lake Michigan water to which motile cells of *Chlamydomonas reinhardi* (food) and prescribed amounts of $CdCl_2$ were added 24 h before use. A food supply of 10^7 cells on Mondays through Thursdays and 3×10^7 cells on Fridays was provided to each population. Except for the few minutes required for daily manipulations, the beakers were kept in a dark incubator (at 18.5 ± 0.5°C) to prevent possible changes in food supply due to cadmium effects on photosynthesis by the algae. The added cadmium contained a constant proportion of ^{109}Cd as a radioactive tracer for the stable cadmium.

Untreated Lake Michigan water was obtained routinely from the Chicago Central Filtration Plant. Some, if not most, of the nonmotile particulate matter was removed by simply allowing it to sediment for a few days. Any zooplankters still remaining were removed by filtering the supernatant water through a net with 10-μm apertures.

Chlamydomonas reinhardi was cultured in petri dishes on agar containing Difco algae broth by subculturing at 2–3-wk intervals. Dense suspensions of motile cells were obtained by flooding several dishes each day with a few millilitres of distilled water and placing them in the dark overnight. The volumes of these suspensions needed for daily preparation of fresh medium were determined by measuring optical density in a Klett-Summerson colorimeter (with red filter) that had been calibrated by hemocytometer counts of heat-killed cells.

Populations were initiated with approximately 20 animals of different ages and were assigned at random to prescribed cadmium levels. Forty populations were studied for 22 wk or, for a few cases at the highest cadmium levels, until they became extinct. In each of two consecutive experiments, four populations were maintained at each of five concentrations of added cadmium: 0, 1, 2, 4, and 8 μg/L in Experiment 1 and 0, 5, 10, 15, and 20 μg/L in Experiment 2. In both experiments, weekly determinations in each population were made of the total number of individuals, mean brood size, and egg–female ratio. This was accomplished by first separating each population from its old medium with a small net, distributing it with some fresh medium into the depressions of spot-test plates, and counting the live animals under a low-power microscope. Then a representative sample of 20% of the total number of live animals was permanently removed for determinations of mean brood size, egg–female ratio, and the numbers of males and ephippial females. The sampling procedure was essentially that described by Marshall (1967) and is believed to be nonselective with respect to the individuals. The only modification of the basic procedure was that most of the sample was obtained by removing all the animals in every fifth depression instead of the first sequence of depressions that would yield the required number.

In Experiment 1, weekly determinations were also made of the dry weight and ^{109}Cd activity of the samples from each population. Dry weight was determined with an electrobalance after drying 24 h at 50°C, and ^{109}Cd activity was determined with a NaI detector–multichannel analyzer system. In addition, all of the aborted eggs, aborted embryos, and shed ephippia that could be found in the used media were counted at the time of each weekly census.

Calculations and Definitions of Symbols

Several statistics were calculated from the basic weekly measurements. Population biomasses were calculated by multiplying the dry weights of the weekly samples by 5. Average individual dry weights were calculated by dividing the dry weights of the samples by the number of individuals in them. The amount of stable cadmium (nanograms) in a sample was calculated by dividing its ^{109}Cd activity (counts per minute) by the specific activity of the added ^{109}Cd/stable Cd spike solution (2.6 counts/min per ng). Determinations of the ^{109}Cd activity in the samples and spike solution were made weekly, using identical counting geometries. The concentrations of cadmium in the dried *Daphnia* samples were calculated by dividing the amount of cadmium in them by their dry weight. Birth rates, b, were calculated from the observed egg–female ratios with the equation developed and recommended by Paloheimo (1974):

$$b = \frac{\ln(1 + E)}{D}, \quad (1)$$

where E is the egg–female ratio and D is the duration of development. As pointed out by Paloheimo (1974), this equation (1) is the same as equation (13) in Edmondson (1968). The egg–female ratio equals the average number of eggs per ovigerous female or brood size (B) times the average proportion of ovigerous females in the population (O). The egg–female ratio included moribund eggs and embryos as well as apparently viable ones, but no attempt was made to correct for prenatal mortality. Thus the calculated birth rate includes stillbirths as well as live ones and is closer to a measure of the egg-laying rate than the live birth rate (J. E. Paloheimo, University of Toronto, personal communication). The duration of development has been found to be primarily a function of temperature and rather independent of nutritional conditions and water

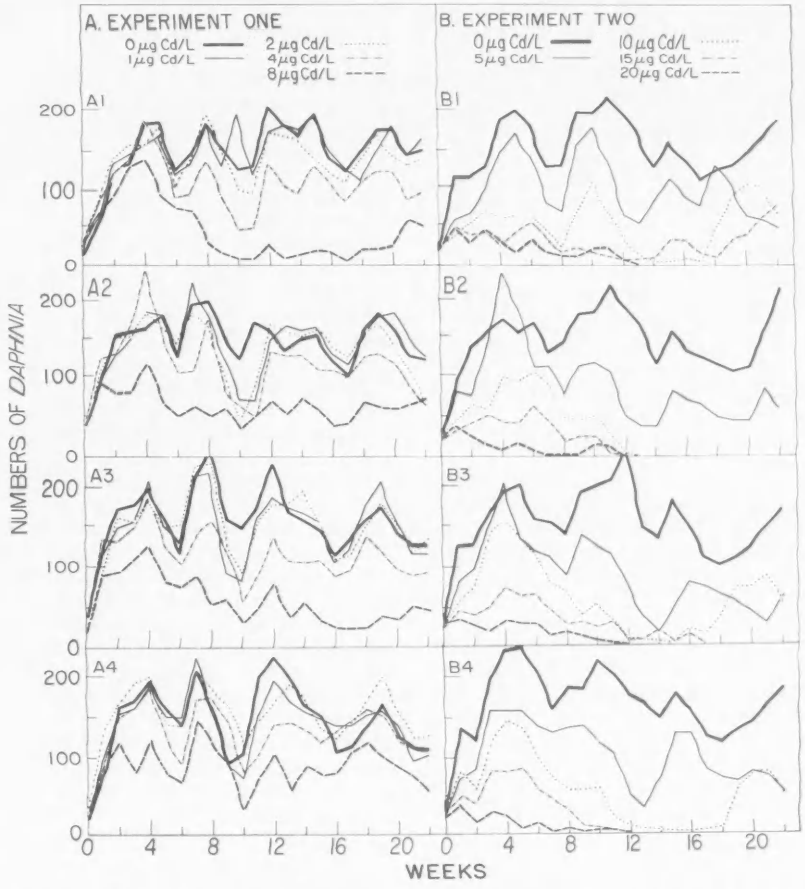

FIG. 1. Effect of chronic cadmium stress on temporal fluctuations of 40 populations of *Daphnia galeata mendotae* in Experiment 1 (replicates A1–A4) and Experiment 2 (replicates B1–B4).

chemistry (Edmondson 1974), or chronic stress (Marshall 1967), so it was assumed to be 2.6 d, the value reported for *D. galeata mendotae* at 20°C by Hall (1964).

Rates of increase, r, were calculated from the weekly census data using the equation

$$r = \frac{\ln N_t - \ln N_{t-7}}{7}, \tag{2}$$

and population death rates, d, were calculated from the equation

$$d = b - r. \tag{3}$$

The calculated death rates account for prenatal as well as postnatal mortality because moribund eggs as well as apparently viable ones were included in the measurements of egg–female ratios. The reciprocal of the average death rate, $1/d$, represents the mean life expectancy reckoned from the beginning of development (i.e. average life span, including development time).

Relative carrying capacity, K, is defined as the long-term average numbers (equilibrium) that are sustained by a population at a given concentration of added cadmium relative to the mean average numbers in the controls, i.e.

$$K = \frac{\bar{N}_{Cd}}{\bar{N}_0}. \tag{4}$$

Coefficients of variability, V, of each population (temporal variability) were calculated with the equation

$$V = \frac{100\, S_N}{\bar{N}}, \tag{5}$$

where S_N is the standard deviation of the weekly censuses and N is the mean.

Results

Numbers and Biomass

The effects of chronic cadmium stress on numbers of individuals and total biomass in the experimental popu-

TABLE 1. Average number of individuals, coefficient of variability (V), and average biomass (mean ± SE) in populations of *Daphnia galeata mendotae* maintained at different concentrations of added cadmium in Lake Michigan water for up to 22 wk (until they became extinct at the highest cadmium concentrations). Values below those underlined are significantly different ($P < 0.05$) from those for controls.

Added Cd (μg/L)	Avg no. individuals	V (%)	Avg biomass (mg dry wt)
0	149±3	29±1	1.09±0.02
1	142±2	30±1	1.07±0.03
2	142±5	28±2	1.08±0.01
4	114±6	32±3	0.99±0.03
5	90±3	50±4	
8	62±9	51±12	0.77±0.07
10	48±6	84±11	
15	23±3	79±14	
20	9±1	131±10	

lations are shown by differences in temporal fluctuations, coefficients of variability, probabilities of extinction, and long-term (22 wk) average values. Temporal fluctuations of the populations (numbers) in the two experiments (Fig. 1) are typical of laboratory *Daphnia* populations and are caused primarily by time lags in adjustment to food supply. The effect of elevated cadmium concentration was a reduction of the maximum and minimum numbers associated with these fluctuations. Fluctuations of different populations in each experiment tended to remain in phase with each other, except at the highest cadmium concentrations. Amplitudes of the fluctuations in the two sets of four control populations in the two experiments were similar, but their period was slightly longer in the second experiment.

None of the populations became extinct in the first experiment, but one at the highest cadmium level came close to extinction after 10 wk and remained very low for the succeeding 10 wk (Fig. 1). Experiment 1 was terminated after 22 wk because plots (not shown) of the cumulative mean numbers for each population had leveled off, indicating an apparent equilibrium about which each population was fluctuating. In the second experiment, several populations at the highest cadmium levels became extinct (Fig. 1). Seven became extinct on weeks 12 and 13, during the declining phase of the second population cycle. These included all four of the populations at 20 μg/L, two at 15 μg/L, and one at 10 μg/L. One of the two remaining populations at 15 μg/L became extinct on week 17, during the declining phase of the third cycle (Fig. 1). Probabilities of extinction can be calculated from these results, although such probabilities depend on the duration of observation as well as cadmium concentration. For 22 wk of observation, however, the probability of extinction is 0 up to 8 μg Cd/L, 0.25 at 10 μg Cd/L, 0.75 at 15 μg Cd/L, and 1.0 at 20 μg Cd/L.

The means of the average numbers of individuals, coefficients of variability, and average biomasses of the populations at each cadmium concentration are shown in Table 1. Mean numbers and biomass decreased with elevated cadmium, while the mean coefficient of variability increased. Although mean numbers, variability, and biomass each show progressive changes with increased cadmium concentrations, the mean values for average numbers and biomass at 1–2 μg/L and for coefficients of variability at 1–4 μg/L are not significantly different from those for the controls. The effect of cadmium on population biomass is less pronounced than that on average numbers because of an increase in average individual dry weight (see Table 4). At 8 μg/L, where average numbers are reduced to 42% of those in the controls, average biomass is reduced only to 71%. The observed increase in the populations' coefficients of temporal variability coincides with the increased probability of extinction and appears to substantiate Leigh's (1975) prediction that greater instability, as measured by coefficient of variability, reduces the expected lifetime of a population (i.e. that increasing variability increases a population's probability of extinction).

Relative carrying capacities calculated for the 32 populations that did not become extinct are plotted against added cadmium concentration in Fig. 2. A linear relationship between relative carrying capacity and cadmium concentration tested is represented by the regression equation and corresponding line (Fig. 2). This relationship is based on the lumped data for both experiments. The data were lumped because a t-test showed that there was no significant difference ($P > 0.05$) between the slopes of the regressions for the two experiments. The linear relationship shown in Fig. 2 predicts that relative carrying capacity would be reduced by 50% at an added cadmium concentration of 7.7 μg/L and by 1% at 0.15 μg/L. In toxicological terms, the median effective concentration (EC50) is 7.7 μg Cd/L and the EC1 is 0.15 μg Cd/L.

TURNOVER

Numerical turnover rates, as represented by the mean rates of birth and death ($\bar{b}$ and $\bar{d}$) in the 32 populations that did not become extinct, showed a progressive increase with elevated cadmium concentrations (Table 2). Weekly fluctuations of the calculated death rates (not shown) closely followed those of the calculated birth rates, while major pulses in the death rate did not cause reductions in the proportion of ovigerous females. Temporal variations in the numbers per capita of aborted eggs and embryos (not shown) tended to parallel those of the calculated death rate and hence provided evidence that the latter were related to prenatal mortality.

Prenatal mortality was by far the most sensitive indicator of cadmium stress observed. This is represented by the linear regression equation and corresponding line in Fig. 3. Each point on this graph represents the total number of aborted eggs and aborted embryos counted in each population during the course of 23 weekly censuses in Experiment 1. The mean number of prenatal deaths at 1 μg Cd/L is 430% higher than that for the controls (30 ± 6 cf. 7 ± 1), and a t-test showed that the difference

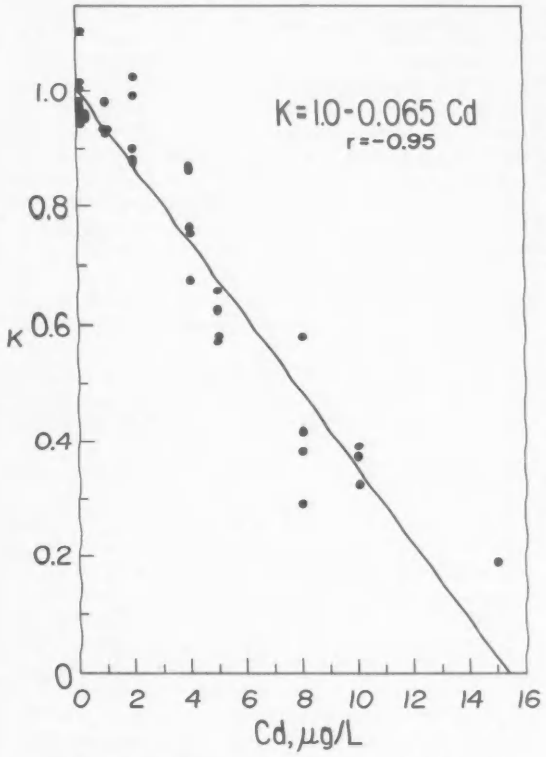

FIG. 2. Effect of chronic cadmium stress on the relative carrying capacity, *K* (relative mean numbers), in populations of *D. galeata mendotae*.

TABLE 2. Number of populations that established stable long-term (22-wk) average numbers at different concentrations of added cadmium and average (mean ± SE) birth and death rates per day for those populations. Values below those underlined are significantly different ($P < 0.01$) from those for controls.

Added Cd (μg/L)	No. of populations	Avg birth rate ($\bar{b}$)	Avg death rate ($\bar{d}$)
0	8	0.087±0.008	0.081±0.007
1	4	0.102±0.022	0.092±0.020
2	4	0.102±0.016	0.094±0.015
4	4	0.149±0.022	0.138±0.022
5	4	0.166±0.024	0.168±0.012
8	4	0.326±0.064	0.306±0.051
10	3	0.242±0.008	0.242±0.009
15	1	0.376	0.372

is highly significant ($P < 0.02$). The linear relationship represented in Fig. 3 predicts that an increase of 0.15 μg Cd/L would approximately double the number of prenatal deaths. The observed number of prenatal deaths probably represents a small fraction of the total number because due to decomposition they become unrecognizable in a matter of hours. Thus the percentage of eggs that failed to complete development cannot be calculated without assuming large corrections for the unobserved prenatal deaths. However, it is quite possible that the total number of live births at 1 μg Cd/L was reduced by approximately 50%, as in *D. magna* (Biesinger and Christensen 1972).

Ovigerous females frequently contained moribund eggs and embryos as well as apparently normal ones in their brood chambers. Although they were not distinguished in determinations of mean brood size or egg–female ratio, the proportion of moribund eggs and embryos appeared to increase with elevated cadmium. While mean brood size increased with cadmium, the average size of the individual eggs decreased. This effect was most noticeable at times when average brood sizes were high and was probably due to a relatively constant brood chamber capacity (volume).

The observed number of postnatal deaths (counted during the first few censuses in Experiment 1) increased with elevated cadmium concentrations and consisted mostly of immature individuals. Cadmium affected the observed postnatal mortality sooner than prenatal; the average number of postnatal deaths observed in the populations above 2 μg Cd/L in the 2nd wk was already

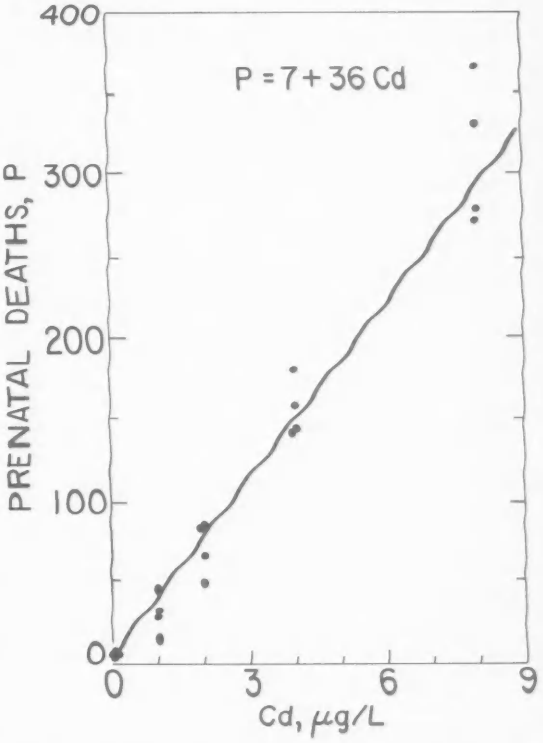

FIG. 3. Effect of chronic cadmium stress on prenatal mortality (observed number of prenatal deaths) in populations of *D. galeata mendotae*.

TABLE 3. Average brood size, ovigerous female–total female ratio, and egg–female ratio (mean ± SE) in populations at different concentrations of added cadmium. Values below those underlined are significantly different ($P < 0.05$) from those for controls.

Added Cd (μg/L)	Brood size ($\bar{B}$)	Ovigerous female:total female ($\bar{O}$)	Egg:female ($\bar{E}$)
0	1.85±0.09	0.14±0.01	0.26±0.03
1	1.97±0.20	0.15±0.02	0.31±0.08
2	1.94±0.22	0.16±0.02	0.31±0.06
4	2.51±0.33	0.19±0.02	0.48±0.09
5	2.85±0.32	0.20±0.01	0.54±0.05
8	3.63±0.46	0.37±0.06	1.43±0.40
10	3.15±0.16	0.28±0.01	0.88±0.03
15	3.95±0.41	0.35±0.04	1.37±0.15
20	4.90±0.25	0.37±0.02	1.81±0.09

TABLE 4. Average dry weight, calculated whole-body cadmium content, and cadmium concentration factor (mean ± SE) for *Daphnia galeata mendotae* in populations maintained at different concentrations of added cadmium in Lake Michigan water for 22 wk. Values below those underlined are significantly different ($P < 0.05$) from those for controls.

Added Cd (μg/L)	Dry wt (μg)	Cd content (μg/g dry wt)	Cd concn factor (10^4)
0	8.13±0.46	≤8[a]	2–8
1	8.24±0.31	17.6±0.36	1.76±0.04
2	8.31±0.27	28.3±0.49	1.41±0.02
4	9.42±0.59	42.8±1.53	1.07±0.04
8	13.90±1.15	51.7±2.67	0.65±0.03

[a]Estimated as explained in text.

significantly higher than that in the controls ($P < 0.05$).

The mean values of average brood size, ovigerous female–total female ratio, and egg–female ratio in the populations at each cadmium level (Table 3) all increased with elevated cadmium, although the means for populations at 1–2 μg Cd/L were not significantly different from those for the controls ($P > 0.05$). These results indicate that the increase in average birth rate (Table 2) resulted from increases in mean brood size and the proportion of ovigerous females in the populations. Since mean brood sizes are rather high, even in the controls, all of the adult females probably had eggs or embryos in their brood chambers most of the time. Thus the observed increase in the proportion of ovigerous females, $\bar{O}$, probably indicates an increase in the proportion of adults in the populations rather than merely an increase in the proportion of adults that were ovigerous females.

INDIVIDUAL ATTRIBUTES

Results pertaining to the average individual in each population include average individual dry weight, average cadmium concentration, and average life expectancy. Mean values of the average dry weights and calculated cadmium content of individuals in the populations at each cadmium concentration in Experiment 1 are shown in Table 4. Average individual dry weights increased with elevated cadmium, but the values at 1–4 μg Cd/L are not significantly different from that for the controls ($P > 0.05$). Using the length–weight relationship for *D. galeata mendotae* published by Burns (1969), the observed average dry weights are found to correspond to lengths of from 0.8 mm in the controls to 1.0 mm in the populations at 8 μg Cd/L. The ages at these lengths vary with growth rate, but *D. galeata mendotae* in this size range are either large juveniles or young adults (Burns 1969; McNaught 1975). The average cadmium content of the animals increased significantly ($P < 0.05$) with each increase in added cadmium concentration from 1 to 8 μg/L (Table 4). Cadmium content of the control *Daphnia* is estimated to be <8 μg/g dry weight. This is obtained by applying a cadmium concentration factor (*Daphnia*–water concentration ratio) of 8×10^4 calculated from published data (Cowgill 1976) to an ambient cadmium concentration in Lake Michigan water of <0.1 μg/L (G. T. Tisue, Argonne National Laboratory, personal communication). The concentration factor for *D. galeata mendotae* in the control populations is probably $>2 \times 10^4$ because it increases from $<1 \times 10^4$ at 8 μg Cd/L to approximately 2×10^4 at 1 μg Cd/L (Table 4). Thus the concentration factor for *D. galeata mendotae* in the control populations is probably between 2×10^4 and 8×10^4. The effects of cadmium on average life expectancy is represented by the modified form of the logistic equation and corresponding line fitted by computer to the calculated mean values shown in Fig. 4. At the higher cadmium concentrations (8–10 μg Cd/L) average life expectancy was reduced to approximately 4 d. This indicates that a major part of the mortality at these cadmium levels occurred very early in life. In view of this and the pronounced effect of cadmium on prenatal mortality, the increase of the proportion of ovigerous females and average individual dry weight suggests that the individuals surviving to maturity may have had nearly normal maximum life spans.

The mean life expectancy at 5 μg Cd/L was reduced to approximately 50% that of the controls, which compares favorably with the 3-wk LC50 of 5 μg Cd/L reported for *D. magna* by Biesinger and Christensen (1972). However, since survivorship in the present study was reckoned from the egg stage, these results indicate that postnatal mortality of *D. galeata mendotae* is not quite as sensitive as that of *D. magna*. One reason for the apparent greater sensitivity of *D. magna* could be a nutritional inadequacy of the food (a pulverized dried grass and trout pellet mixture) used by Biesinger and Christensen (1972), as shown by Winner et al. (1977) for the effect of such food on chronic toxicity of copper to *D. magna*. Winner and Farrell (1976) showed that with *Chlamydomonas reinhardi* as food *D. magna*, *D. pulex*, *D. parvula*, and *D. ambigua* did not differ significantly in their susceptibility to a chronic copper stress, so *D. galeata mendotae* probably does not differ greatly from *D. magna* in its intrinsic sensitivity to cadmium.

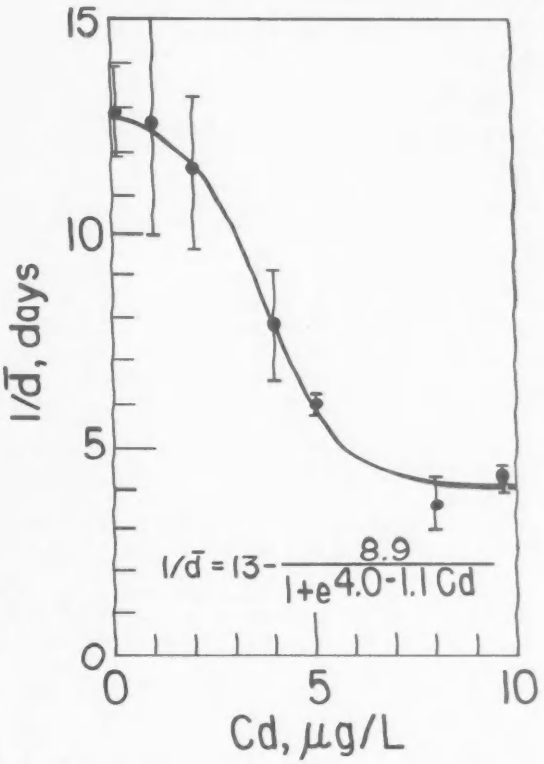

FIG. 4. Effect of chronic cadmium stress on average life expectancy, $1/\bar{d}$, of *D. galeata mendotae* ($\bar{d}$ is average population death rate). Vertical bars represent ±SE.

Discussion

Perhaps the most important generalization to be drawn from this study is that the effects of chronic cadmium stress on *Daphnia* populations are similar to those observed in response to chronic radiation stress (Marshall 1966) or frequent removal of fixed percentages of the newborn (Slobodkin and Richman 1956). Cadmium stress reduced the populations' average numbers and biomass, while it increased population variability, probability of extinction, turnover rate, and the proportion of ovigerous females in the populations. Compensatory adjustments in the populations that did not become extinct allowed the birth rates to increase sufficiently to match the increased death rates. These compensatory adjustments consisted of increased mean brood size and an increase in the proportion of ovigerous females in the populations. The increased brood size was primarily a result of increased food supply per individual because of the reduced standing crop (numbers and biomass). One reason for the decreased standing crop would have been the increased maintenance costs resulting from prenatal and early postnatal mortality. The effects of chronic cadmium stress on populations of *D. galeata mendotae* are also similar to the effects of different kinds of stress in other animal populations. Nicholson (1954, 1957) observed similar stress effects and compensatory reactions in experimental populations of the sheep blowfly; he also reviewed the evidence from vertebrate population studies and concluded that the capacity of self-adjustment of populations to stress is automatic and a product of evolution. More specifically, Nicholson (1957) showed that if a stress increases the mortality rate of a certain age-class, compensatory adjustments will result in an increase in the age-class immediately preceding it. This appears to have occurred in the present study; i.e. increased egg mortality resulted in a compensatory increase in the proportion of egg-bearing females.

The high reproductive capacity of *Daphnia* populations is required to compensate for mortality from many stresses in the natural environment, but in the laboratory most of this capacity may be available to compensate for a single experimental stress. Even in the laboratory, however, there is a limit to this compensatory capacity. In the present study, decreased population density allowed the birth rate to match the increased death rate only up to a point. The maximum tolerable cadmium concentration (that which reduced the carrying capacity to zero) was probably determined by the maximum attainable birth rate, b_{max}, which is essentially equivalent to the maximum rate of increase, r_{max}, under the given conditions. It is probably not coincidental, therefore, that the average birth rates in populations near zero carrying capacity (0.24–0.38 /d) were equal to or greater than the maximum birth rates and rates of increase reported by Hall (1964) for *D. galeata mendotae* at comparable temperatures. In the laboratory at 20°C under a wide range of food levels, r_{max} ranges from 0.22 to 0.34 /d (Hall 1964). In Base Line Lake an apparent predation rate of just over 0.25 /d was observed in a *D. galeata mendotae* population that was decreasing very slowly, which indicated a turnover time of about 4 d (Hall 1964).

Three conclusions of practical importance in assessing the ecological significance of the results are as follows: (1) because of compensatory increases in the proportion of ovigerous females and mean brood size, the cadmium sensitivity of population attributes is considerably less than that of the most sensitive attribute of individuals or life history, namely prenatal mortality; (2) there is no apparent threshold for the effects of chronic cadmium stress on population attributes such as carrying capacity; (3) an increase of 0.15 μg Cd /L in Lake Michigan, barring synergistic interaction with other factors in the lake, would probably not have a detectable effect on the average numbers or population dynamics of *D. galeata mendotae*. The first conclusion is based on the results' consistency with the hypothesis that compensatory adjustment to stress is an innate capacity of animal populations. The second conclusion is based on the observed linear relationship between carrying capacity and cadmium and the general observation that thresholds do not exist for many types of chronic stress (Woodwell 1975). The third conclusion is based on the same linear relationship, but it is not conservative without the quali-

fication barring synergistic interaction of cadmium with factors that were not investigated or accounted for in this study.

Synergistic interactions and indirect effects of cadmium on *Daphnia* populations via the food supply (quality or quantity) and fish predation, however, are quite likely to occur in the natural environment. The sensitivity of *Daphnia* to chronic copper stress, for example, is increased with food of reduced quality (Winner et al. 1977). A reduction of the quantity of food by cadmium would likewise compound its effect on *Daphnia* populations. This indirect effect was eliminated in this study by preventing the growth of the algae. In the natural environment, however, light will tend to interact synergistically with cadmium if the latter, in addition to affecting the *Daphnia* directly, simultaneously reduces the growth of phytoplankton. Fish predation may interact synergistically with cadmium stress because it is selective for the larger sizes of zooplankters (Hall et al. 1976). Since an increase in the proportion of the larger size-classes (ovigerous females) is a compensatory reaction to cadmium stress, fish predation would counteract a *Daphnia* population's compensatory reaction to cadmium. In contrast to size-selective exploitation by planktivorous fish, nonselective exploitation has been found to interact antagonistically with chronic cadmium stress in laboratory copepod populations (Hoppenheit 1975) and with chronic radiation stress in *Daphnia* populations (Marshall 1967).

Other factors in the natural environment with which cadmium might interact synergistically include interspecific competition, physical conditions, chemical factors, and other pollutants. *Daphnia galeata mendotae* probably has a competitive advantage over the smaller cladocerans in Lake Michigan, but when the population of *D. galeata mendotae* is severely reduced by alewife (*Alosa pseudoharengus*) predation, the smaller species proliferate because of reduced competition (Wells 1970). If the smaller species are less sensitive to chronic cadmium stress, they might gain a competitive advantage over *D. galeata mendotae* with increased cadmium. With increased radiation stress in mixed populations of *D. magna* and the smaller *D. pulex*, for example, the normal competitive advantage of *D. magna* is gained by *D. pulex* because the latter is less sensitive to radiation (Marshall 1969). The effect of temperature upon the toxicity of chemicals to aquatic organisms has received more attention than that of any other physical factor, yet there is little information on its effect on heavy-metal toxicity and none on its effects on chronic cadmium stress (Cairns et al. 1975). The chemical species of heavy metals that exist in the natural environment may be different from those studied in laboratory toxicity studies if insufficient time is allowed for chemical speciation of the added metal in the test media before use (Allen et al. 1976). Although the 24 h allowed for chemical speciation in the present study is considerably longer than the time allowed in most studies, time may not be critical for additions of Cd^{+2} ion. The literature indicates that most of the cadmium in natural waters is in solution (Fleischer et al. 1974), and in waters of pH and hardness comparable with that of Lake Michigan, Cd^{+2} is probably by far the predominant species (Davies 1976). Changes in the relative concentrations of other pollutants in Lake Michigan might also modify the effects of increased cadmium concentrations on *D. galeata mendotae* populations.

Although the present study on isolated populations of *D. galeata mendotae* is more holistic than studies on isolated generations (cohorts), it is less holistic than experiments with natural communities. The usefulness of in situ controlled environmental pollution experiments in assessing the effects of copper on a marine plankton community was shown by Reeve et al. (1976), who concluded that the zooplankton was the component most affected. The results of an in situ study of cadmium stress in a Lake Michigan zooplankton community showed that cadmium enrichments as low as 5 μg/L caused large and highly significant effects on functional and structural attributes of the community within 9 d, while the effects of enclosure (up to 15 d) were mostly insignificant (Marshall and Van Reken 1977). This study indicated that in situ experiments somewhat longer than 15 d may be feasible, but long-term experiments (up to several months) are not feasible in the Great Lakes. Therefore future determination of the relationships between short- and long-term effects of stress in laboratory populations of zooplankters that occur in these lakes and the same relationships in small, experimental lakes (Schindler and Fee 1974) will be useful in assessing the long-term significance of the results of in situ experiments in large lakes.

Acknowledgments

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Fish Cell Culture: Properties of a Cell Line from the Sheepshead, *Archosargus probatocephalus*

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LAW, W. M., R. D. ELLENDER, J. H. WHARTON, AND B. L. MIDDLEBROOKS. 1978. Fish cell culture: properties of a cell line from the sheepshead, *Archosargus probatocephalus*. J. Fish. Res. Board Can. 35: 470–473.

A cell line designated SHF-1 was established from fin tissue of the sheepshead, *Archosargus probatocephalus*. Cells were fibroblastlike and grew best at 26°C in Leibovitz (L-15) medium containing serum supplements and a final sodium chloride concentration of 0.150 M. The 2*N* number of primary sheepshead cells was 48 but the SHF-1 line at passage levels above 11 was heteroploid. SHF-1 cells did not replicate any of seven viruses tested. Species confirmation of SHF-1 was accomplished by the cytotoxic antibody test and was attempted with isoelectric focusing of lactic dehydrogenase isoenzymes. Plating efficiency and freeze viability studies were also performed.

Key words: fish cell culture, sheepshead, *Archosargus probatocephalus*, microbiology

LAW, W. M., R. D. ELLENDER, J. H. WHARTON, AND B. L. MIDDLEBROOKS. 1978. Fish cell culture: properties of a cell line from the sheepshead, *Archosargus probatocephalus*. J. Fish. Res. Board Can. 35: 470–473.

Une lignée de cellules, nommée SHF-1, a été établie à partir du tissu de nageoire du Malachigan *Archosargus probatocephalus*. Les cellules, apparentées aux fibroblastes, ont eu une une croissance maximale à 26°C, dans un milieu de Leibovitz (L-15) additionné de sérum et dont la concentration finale de chlorure de sodium était de 0,150 M. Les cellules mères diploïdes possédaient 48 chromosomes, mais, après 11 générations, la lignée SHF-1 était devenue hétéroploïde. Il n'y a pas eu réplication des sept souches virales introduites dans les cellules de la SHF-1. La spécification de la SHF-1 a été confirmée par le test des anticorps cytotoxiques et elle a été tentée par l'électrofocalisation d'isoenzymes du groupe de la laticodéshydrogénase lactique. Des études ont aussi été menées sur leur capacité de former des plages et sur leur viabilité en période de congélation.

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MARINE fish tissue cultures have been documented with increased frequency during the past 16 yr. The more recent reports describe cell lines from the Atlantic salmon (AS) (Nicholson and Byrne 1973), Omaka (Lee and Loh 1975), silver perch (SP-1) (Wharton et al. 1977), and lumpfish (Li and Clyburne 1977).

We have concentrated on the development of cell cultures from fish of the Gulf of Mexico to aid the isolation and identification of unrecognized marine fish viruses. This new cell line from the sheepshead, *Archosargus probatocephalus*, has been subcultured for 2 yr and is presently in passage 54.

Materials and methods — The SHF-1 line was initiated from a single explant culture of caudal fin tissue of an adult male sheepshead. We used Leibovitz medium (L-15) 80%, with 15% fetal bovine serum, 5% human serum, and 1% of 1.3 M NaCl solution. Cells were grown in 25-cm² Falcon flasks at 26°C and subcultured with trypsin–versene® (ATV) (Madin and Darby 1958).

The procedures used to characterize SHF-1 cells were described or referenced in an earlier publication (Wharton et al. 1977). Studies included growth curves at 18, 26, and 32°C, karyological analysis of primary sheepshead fin cells and three passages (11, 30, 50) of the SHF-1 line, virus susceptibility of SHF-1 cells to four fish, one amphibian, and two mammalian viruses (IPN, CCV, SVC, FV-3, EEE, VSV, lymphocystis), viability of SHF-1 cells following storage in liquid nitrogen, species identification of SHF-1 cells by the cytotoxic antibody dye exclusion test and by isoelectric focusing of lactic dehydrogenase (LDH) isoenzymes, and the plating efficiency of SHF-1 cells. All procedures of characterization of SHF-1 cells were performed between passages 10 and 50.

Results — SHF-1 cells have a fibroblastlike morphology (Fig. 1). When 1×10^5 cells/25 cm² surface area were seeded into L-15 growth media, monolayers were formed in 2–3 d, remained viable for approximately

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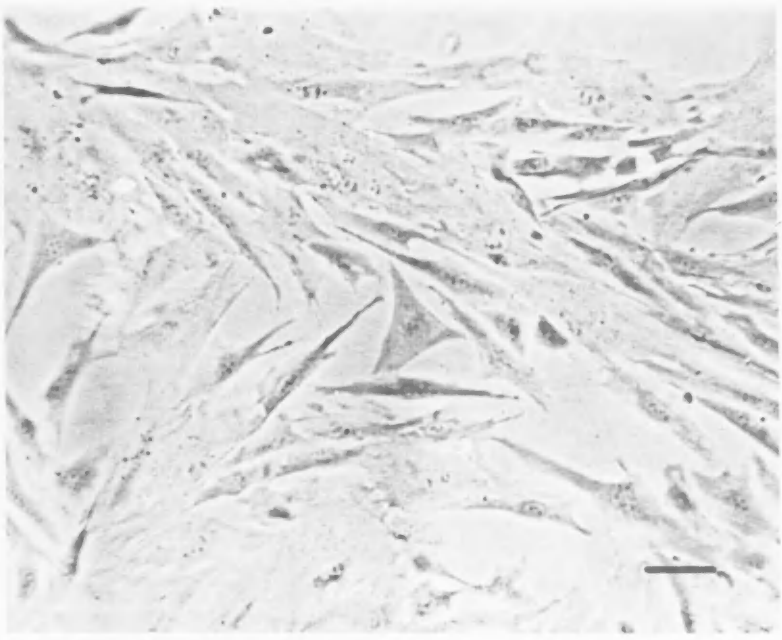

FIG. 1. Unstained SHF-1 cells under phase-contrast microscopy. Bar = 40 μm.

TABLE 1. Karyological analyses of primary and subcultured sheepshead cells.

Passage level	No. cells counted	$\bar{x} \pm$ SD[a]	Modal no.
1	503	47.7±0.2	48
11	101	44.2±0.3	44
30	50	41.4±0.2	44
50	101	44.6±0.3	47

[a]Mean (untransformed data); standard deviation (root square transformation).

2 wk, then died because of the acidic condition of the media. Figure 2 illustrates the growth of SHF-1 cells (passage 27) at three incubation temperatures. At 26°C, cell inocula of 9.5×10^4 cells/25 cm^2 surface area produced confluent monolayers (approximately 1.2×10^6 cells/25 cm^2 surface area) in 3 d, whereas cells incubated at 32°C never reached confluency and declined in number after 6 d. SHF-1 cells incubated at 18°C required 5 d to reach confluency.

A karyological analysis of primary sheepshead fin cells was conducted, since no previous determination of the 2*N* number of *A. probatocephalus* was available (Roberts 1964; Denton 1973). Passage 1 sheepshead cells contained a 2*N* value of 48 and a mean of 47.2 (Table 1). The modal number decreased in subsequent passages (11 and 30) to 44 and by passage 50 had increased to 47. Primary cells with 48 chromosomes usually contained 43 telocentric and acrocentric chromosomes, 4 submetacentrics, and 1 small metacentric chromosome. The morphology of SHF-1 cells at passage 50 most often included 43 acrocentric and telocentric chomosomes and 4 submetacentric chromosomes. A karyotype of P-50 chromosomes is shown in Fig. 3.

SHF-1 cells did not support the replication of the seven viruses used in this study. The failure of each virus to replicate in SHF-1 cells was not due to an inactive viral preparation nor the temperature of incubation, since each virus replicated in susceptible cells incubated at temperatures identical to those used for SHF-1 cells.

Freeze viability studies indicated that SHF-1 cells (passages 34, 43, 44) frozen (−196°C) in L-15 growth media containing 5% dimethylsulfoxide averaged 87% viability over a 30-d period. Viability was low (average 33%) when 10% glycerol was employed.

The plating efficiency of SHF-1 cells in L-15 medium was tested at passages 27, 28, and 31. In these studies, the efficiency ranged from 24 to 35%.

The results of the cytotoxic antibody dye exclusion test are given in Table 2. Exposure to rabbit antisheepshead homogenate serum and complement killed 69.7% of SHF-1 cells, 38.8% of BB (channel catfish) cells, and 34.6% of FHM (fathead minnow) cells. Antiserum tested without complement killed 14.6% of SHF-1

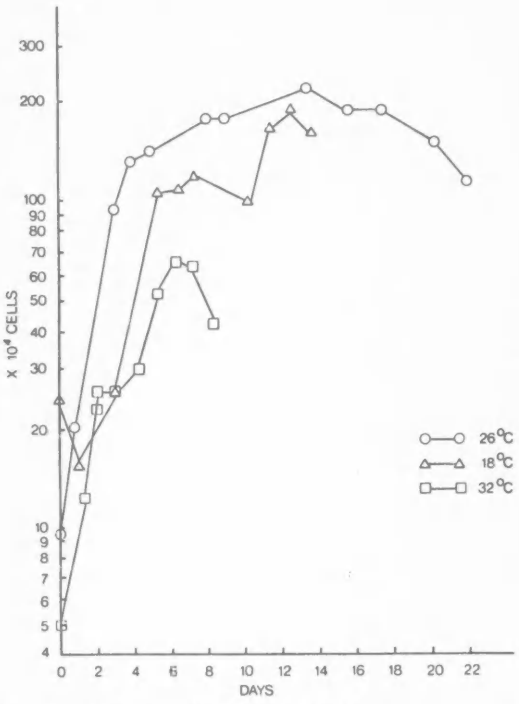

FIG. 2. Growth of SHF-1 cells (P-27) at temperatures of 18, 26, and 32°C.

TABLE 2. Effect of rabbit antisheepshead homogenate serum on SHF-1, BB, and FHM cells. T = average of two tubes receiving rabbit antisheepshead homogenate serum, complement, and cells; CC = complement control tube; SC = serum control tube.

Cell type/ passage	Initial cell × 10³ concn	Mean viable cells × 10³			% of initial cell concn		
		T	CC	SC	T	CC	SC
SHF-1 P-46	249	75.4	212.6	202.2	30.3	85.4	81.2
BB P-173	310	190.5	288.6	292.5	61.2	93.1	94.4
FHM P-107	275	179.8	231.4	257.4	65.4	84.1	93.6

cells, 6.9% of BB cells, and 15.9% of FHM cells. Normal rabbit serum with complement killed 18.8% of SHF-1 cells, 5.6% of BB cells, and 6.4% of FHM cells.

Primary sheepshead and SHF-1 cells were also characterized by isoelectric focusing of LDH isoenzymes. Analysis of four separate cultures of primary sheepshead cells (P-2) gave two LDH bands with isoelectric points (pI) of 6.98 and 7.11, respectively. SHF-1 cells as passage 45 contained a single LDH band (isoelectric point of 7.02) which was consistent in eight individual analyses.

Discussion — The sheepshead cell line appears to be a stable culture that is simple to maintain. It was not surprising that this line grew best at 26°C, since this temperature is close to the preferred temperature of the animal. Although the cell line was adapted to grow in

FIG. 3. Tentative arrangement of SHF-1 chromosomes, P-50. $2N = 47$. Bar = 7 μm.

L-15 medium, it will replicate in other media. Studies not reported here demonstrated that either minimal essential medium containing Hank's balanced salt solution or medium 199 supplemented with additional NaCl could replace L-15 for cell growth. We routinely use L-15 medium because cell growth does not produce a rapid pH shift toward the acid side and because the medium is not affected by the CO_2 tension of the atmosphere of the flask.

Primary cell growth required low concentrations of human serum (1–5%) but was inhibited by 10% human serum. By passage 10, SHF-1 cells had no nutritional requirement for human serum and it was deleted in all further subcultivations.

In contrast to our results with SP-1 cells (Wharton et al. 1977), subcultured SHF-1 cells contained fewer chromosomes than primary sheepshead cells. Possibly a Robertsonian translocation occurred in vitro producing a 2*N* number that was less than the donor species. We are currently studying G and C banding of SHF-1 cells in the hope that such techniques will allow correct pairing of fish chromosomes.

Smith and Taylor (1972) reported natural lymphocystis infection of sheepshead but we were not able to infect SHF-1 cells with lymphocystis virus isolated from *Micropogon undulatus* and *Bairdiella chrysura* lesions. The systematic study of the other six viruses showed that these agents did not replicate in SHF-1 cells.

The cytotoxic antibody test used in this study serologically identified SHF-1 cells. The test was positive but about 30% of SHF-1 cells survived the effects of rabbit antisheepshead homogenate, possibly indicating that the antibody titer was low or that the prepared homogenate was not nearly as effective an antigen as whole cells or low passage SHF-1 cells.

We have investigated isoenzyme analysis of fish cells in culture as an adjunct to the cytotoxic antibody test. The results are promising but isoenzyme patterns can change as cells are cultured from primary to established status, indicating the need for additional developmental work. Our earlier study (Wharton et al. 1977) showed that the LDH profile of a cultured fish line can be quite diverse. We speculate that for the purpose of cell line characterization, analysis of several isoenzymes would provide the degree of differentiation needed for routine cell identification or detection of contamination by other cell lines.

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Chill Stowage and Development of Rancidity in Frozen Pacific Herring, *Clupea harengus pallasi*

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BILINSKI, E., R. E. E. JONAS, AND Y. C. LAU. 1978. Chill stowage and development of rancidity in frozen Pacific herring, *Clupea harengus pallasi*. J. Fish. Res. Board Can. 35: 473–477.

Freshly caught Pacific herring, *Clupea harengus pallasi*, were stowed in ice or refrigerated seawater (RSW) at −0.8°C for 0, 2, and 4 d and were then filleted and stored frozen

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(−28°C) for up to 11 mo. The development of rancidity was determined using the peroxide value and the thiobarbituric acid number. During the chill stowage before freezing, the lipids from the flesh were not subjected to any significant oxidation. In Cryovac-vacuum-packed fillets the rancidity remained at low levels during the duration of frozen storage. If oxygen was present during frozen storage, chill stowage accelerated the development of rancidity and this effect was more pronounced in the case of RSW than ice, especially after 4 d of stowage.

Key words: Pacific herring, *Clupea harengus pallasi*, rancidity, icing, refrigerated seawater, frozen storage

BILINSKI, E., R. E. E. JONAS, AND Y. C. LAU. 1978. Chill stowage and development of rancidity in frozen Pacific herring, *Clupea harengus pallasi*. J. Fish. Res. Board Can. 35: 473–477.

Des harengs du Pacifique, *Clupea harengus pallasi*, fraîchement capturés furent maintenus dans de la glace ou de l'eau de mer réfrigérée (EMR) à −0.8°C pendant 0, 2 et 4 jours. On les a ensuite filettés et entreposés à l'état congelé (−28°C) pendant des périodes allant jusqu'à 11 mo. Nous avons mesuré le progrès de la rancidité à l'aide de la valeur de peroxyde et de l'indice d'acide thiobarbiturique. Lors de l'entreposage à froid précédant la congélation, les lipides de la chair ne subissent pas d'oxydation à un degré significatif. Dans des filets entreposés dans un emballage Cryovac sous vide, la rancidité se maintient à de bas niveau durant toute la durée de l'entreposage congelé. S'il y a de l'oxygène présent durant l'entreposage congelé, l'arrimage à froid accélère le développement de la rancidité. Cet effet est plus prononcé dans le cas de l'EMR que dans celui de la glace, surtout après un arrimage de 4 jours.

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THE herring fishery, in both the Atlantic and Pacific regions, is presently evolving toward an increased utilization of the catch for production of human food rather than for reduction to oil and animal feed. The preservation of herring before freezing or processing as food is made difficult by its rapid spoilage and physical fragility. There is an existing trend to use refrigerated seawater (RSW) for this purpose, in addition to the traditional icing (Roach et al. 1961, 1967; Gakichko et al. 1962; Karsti and Blokhus 1966; Hulme and Baker 1977). During frozen storage the deterioration of the quality of herring is primarily due to the development of oxidative rancidity (Banks 1952) but understanding of the effects of icing and RSW on the oxidative changes is still fragmentary (Hansen et al. 1970). The purpose of this investigation was to compare the effects of the stowage in ice and RSW on the development of rancidity during frozen storage of Pacific herring, *Clupea harengus pallasi*.

Materials and methods — Pacific herring were caught in early November by a commercial seiner in the Trincomali Channel, B.C., and transferred directly from the seiner to the R.V. *Investigator No. 1*. Stowage of the whole herring in ice or RSW, filleting, freezing, and the initial stages of frozen storage were carried out on board ship. The procedures for stowage of fish in experimental RSW tanks or in ice and for freezing were previously described (Bilinski et al. 1977). For filleting, herrings were beheaded and eviscerated and a split was made along the central bone in such a way that two fillets were still connected in the back (butterfly fillet). The control fish (0 d) were filleted and frozen less than 3 h after catch. Other fish were held in melting ice or RSW (−0.8°C) for 2 or 4 d before filleting and freezing. After freezing, fillets were vacuum packed in barrier bags (Cryovac) using the Multivac sealer or placed in air permeable plastic bags. Each bag contained six fillets. During frozen storage the fillets were held at −28°C. The fillets had an average weight of 110.91 g (SE = 5.18, $n = 18$). The fat and water content of the fillets were 14.40% (SE = 0.71, $n = 18$) and 65.04% (SE = 0.56, $n = 18$), respectively.

For the chemical determinations half of a partially frozen butterfly fillet was homogenized using the omnimixer with the cup immersed in an ice bath. Total fat was extracted from the homogenate (30 g) by the method of Bligh and Dyer (1959). The peroxide values (PV), indicating the milliequivalents peroxide per kilogram fat, was determined on aliquots of the chloroform–fat extracts by the standard method of AOCS (1976). This method has been adopted for microdeterminations (200–500 mg fat) by conducting the titration under nitrogen with a Gilmont micrometer syringe (capacity 2.0 mL) immersed in the reaction mixture. Thiobarbituric acid test (TBA) was conducted according to the method of Vyncke (1970) and the results are expressed as TBA number, signifying milligrams malonaldehyde per kilogram muscle (Sinnhuber and Yu 1958).

In tests for the incidence of rancid off-flavor, one fillet of each fish was used for organoleptic evaluation and the other for the determination of PV and TBA number. Fillets, wrapped in aluminum paper, were steamed for 10 min before being subjected to taste-panel evaluation. In most cases the onset of rancid off-flavor could be detected more readily in the red than in the white muscle.

For the statistical comparison of the analytical results, Student's *t*-test was used.

Results and discussion — In preliminary tests, the levels of PV and TBA coinciding with a discernible rancid off-flavor of the flesh were estimated using the frozen fillets. In 28 fish showing a very low incidence of rancid off-flavor (10% of samples) the average PV

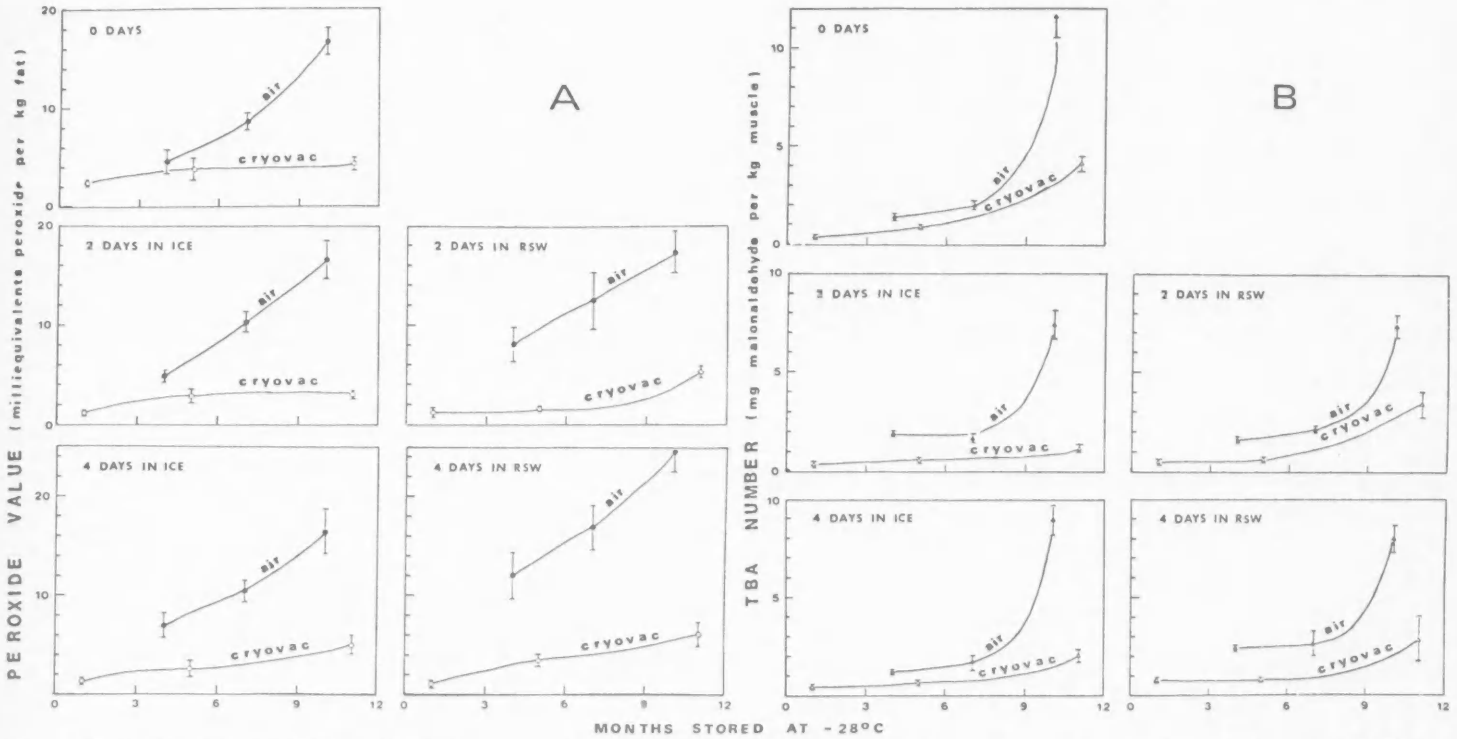

FIG. 1. Development of rancidity in Pacific herring, *Clupea harengus pallasi*, fillets during frozen storage, measured by peroxide value (A) and thiobarbituric acid number (B). Each point indicates the mean value for six fish; SE is shown by the vertical line.

amounted to 4.62 (SE = 0.39). On the other hand, in 30 fish with a high incidence of rancid off-flavor (70%) the PV averaged 18.39 (SE = 1.03). For the same two groups TBA number amounted to 2.71 (SE = 0.32) and 8.55 (SE = 0.54), respectively.

The results of the experiments on the development of rancidity during frozen storage of vacuum-packed and air-exposed fillets are shown in Fig. 1. In vacuum-packed fillets, PV remained in all cases at low levels (<5), though it showed a tendency to increase with time. During the duration of frozen storage there was no statistically significant difference in PV of vacuum-packed fillets subjected to different treatments before freezing. The TBA number also remained at comparatively low levels (≦4) and showed no consistent difference in relation to the treatment of fish before freezing.

In fillets exposed to air during frozen storage the oxidation of lipids was apparent from the progressive increases in PV. In herring held for 2–4 d in RSW or for 4 d in ice PV was significantly higher in air-exposed than in vacuum-packed fillets after 4 mo. In fish frozen immediately after catch or kept for 2 d in ice before freezing the large increase in PV occurred later. After 10 mo in the presence of air PV reached unacceptable high levels in all cases. The TBA number showed a very large increase in fillets kept for 10 mo in the presence of air but this test failed to reflect the progressive lipid oxidation occurring during the earlier stages of frozen storage.

Our results show consistently low levels of PV and TBA in vacuum-packed fillets after 1 mo of frozen storage, which strongly suggests that lipids were not subjected to any significant oxidation when herring was held for up to 4 d in ice or RSW. This agrees with the study of Sigurdsson (1947), who found that PV of herring showed little increase until the 5th d of stowage at 0°C. Karsti and Blokhus (1966) found that PV remained at low levels and there was no rancid off-flavor in herring kept for 3 d in ice or in chilled brine (3–10% NaCl), but after 6 d and longer periods of chill stowage, the oxidative deterioration took place and it occurred at a faster rate in iced than in brined fish. Meyboom and Van Pel (1965) found that a prolonged stowage of herring in ice gave rise to rancidity in the oil of canned fish but the rancidity did not develop when RSW was used. Thus, during longer periods of chill stowage the rancidity apparently develops faster in iced herring than in fish totally immersed in water, due to the reduced access of air in the latter case. However, the chill stowage of food herring should not exceed 2–3 d in view of the rapid autolytic and bacterial spoilage. If oxygen was largely excluded during frozen storage the development of rancidity was not influenced by stowage of herring in ice or RSW. But if oxygen was present chill stowage accelerated the oxidative changes in frozen herring and this effect was more pronounced in the case of RSW than ice. In earlier studies, Banks (1938) found that a 2-d stowage of herring in ice showed no effect on PV during frozen storage. Gakichko et al. (1962) and Piskariew (1967) obtained evidence that holding of herring before freezing in RSW increased PV in frozen fish. Pielichowski and Nodzynski (1974) found that during frozen storage the rancid off-flavor developed faster in herring stowed for 3 d in ice-chilled seawater than in fish frozen directly after catch or chilled for only 1 d. Atlantic mackerel, *Scomber scombrus*, is also very susceptible to rancid spoilage, and the delay of freezing accelerates oxidative changes during frozen storage (Ke et al. 1976; Hardy and Smith 1976).

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Comparaison des échanges branchiaux de Cl^- en eau douce chez *Salmo gairdneri* in vivo et in vitro sur la tête isolée perfusée

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Les flux entrants de Cl^- sont identiques in vivo et in vitro à condition que les branchies soient irriguées extérieurement pendant la préparation de la tête isolée. Dans une telle préparation on observe une absorption nette de Cl^- par les branchies. Lorsque la préparation n'est pas irriguée, le flux entrant est réduit de 66% et le bilan des échanges de Cl^- devient négatif.

PAYAN, P., P. PIC ET G. DE RENZIS. 1978. Comparaison des échanges branchiaux de Cl^- en eau douce chez *Salmo gairdneri* in vivo ou in vitro sur la tête isolée perfusée. J. Fish. Res. Board Can. 35: 477–479.

The Cl^- influxes are identical in vivo and in vitro providing that the gills are externally irrigated during the preparation of the isolated head. A net uptake of Cl^- is observed. When no irrigation is used the Cl^- influx is reduced by 66% and Cl^- is lost by the preparation.

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CHEZ les poissons téléostéens d'eau douce, les branchies sont le siège d'une absorption active de Na^+ et Cl^- ; les deux ions sont absorbés indépendamment en échange d'ions endogènes de même signe, H^+ ou NH_4^+ et HCO_3^- (Krogh 1939 ; Kerstetter et Kirschner 1972 ; Maetz et al. 1976). Dans le but de préciser ces mécanismes d'échanges ioniques, diverses techniques de perfusion de branchies ont été utilisées permettant de modifier à volonté la composition ionique du milieu intérieur et d'étudier l'effet des hormones ou de substances pharmacologiques. Ces différentes tentatives ont abouti le plus souvent à des préparations présentant des flux nets de Na^+ et Cl^- négatifs. La technique de la tête isolée perfusée de truite arc-en-ciel, *Salmo gairdneri*, mise au point par Payan et Matty (1975) a permis d'obtenir in vitro des échanges de sodium et d'ammonium de même intensité que ceux observés chez l'animal in vivo (Payan et al. 1975). De plus en présence d'adrénaline dans le milieu de perfusion, on observe une absorption nette de Na^+ par la préparation. Ce travail a été réalisé dans le but de préciser si cette technique présente pour l'étude des échanges de Cl^- les mêmes avantages que pour le Na^+.

Matériel et méthodes — Des truites arc-en-ciel (*Salmo gairdneri*) d'un poids moyen de 180 g ont été utilisées pour mesurer les flux unidirectionnels de Cl^- à travers les branchies lors de deux types d'expériences : in vivo et in vitro.

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La mesure des flux chez l'animal entier a été effectuée sur des truites maintenues en aquarium de mesure depuis au moins 24 h. La durée de la période de mesure est de 2 h. La technique est celle décrite par de Renzis (1975) sauf que les animaux ne sont pas munis d'un cathéter urinaire ; en conséquence le flux sortant est un flux sortant global urinaire et branchial.

Les expériences in vitro ont été réalisées selon la technique de la tête isolée et perfusée de truite précédemment décrite (Payan et Matty 1975). La perfusion des branchies a été réalisée à débit constant (300 mL/h) (Girard 1976) avec une solution de perfusion de même composition que celle utilisée précédemment et contenant 10^{-6} mol/L d'adrénaline pour assurer une bonne irrigation. Chaque expérience comporte une période de mesure de 15 min succédant à un rinçage des voies circulatoires par la solution de perfusion pendant 15 min permettant d'éliminer le sang restant dans la tête. La détermination du flux entrant de Cl^- se fait en suivant la disparition du ^{36}Cl dans le milieu externe. Le calcul des flux unidirectionnels est le même que pour le Na^+ (Payan et al. 1975). Dans une série d'expériences les branchies ont été irriguées pendant la durée de l'opération (5 à 10 min). Cela consiste à arroser abondamment (1 L/min) les branchies avec de l'eau douce au moyen d'un tuyau positionné dans la bouche de l'animal face à l'appareil branchial. Dans une autre série d'expériences, conformément à la technique d'origine, les branchies ne sont pas irriguées.

Toutes les expériences in vivo et in vitro on été réalisées à 17°C en utilisant un milieu externe composé d'eau douce additionnée de NaCl (concentration finale 1 mM).

Résultats et discussion — Les valeurs moyennes ±ES ($n = 8$) des flux de Cl^- mesurés in vivo sont :

Flux entrant	Flux sortant	Flux net
25.0 ± 4.66	19.9 ± 5.82	+5.1 ± 6.50

Les flux sont exprimés en $\mu Eq \cdot h^{-1} \cdot 100\ g^{-1}$. Les flux entrants sont comparables à ceux obtenus par Kerstetter et Kirschner (1972) chez la même espèce. Les animaux présentent un flux net dont la valeur positive n'est cependant pas statistiquement significative ; ceci indique que chez ces animaux la balance du Cl^- est équilibrée car le flux entrant branchial compense le flux sortant total. Cependant, si l'on tient compte des valeurs de l'excrétion urinaire de Cl^- rapportées antérieurement chez *Salmo gairdneri* (Holmes et Stainer 1966) (3.3 à 3.5 $\mu Eq \cdot h^{-1} \cdot 100\ g^{-1}$), le flux net d'absorption branchiale dans nos expériences pourrait atteindre environ 8.5 $\mu Eq \cdot h^{-1} \cdot 100\ g^{-1}$.

Les effets de l'irrigation externe des branchies sur la valeur moyenne ±ES des flux de Cl^- mesurés sur la tête isolée et perfusée sont :

	Flux entrant	Flux sortant	Flux net
Sans irrigation des branchies ($n = 9$)	8.7±0.82	23.9±5.51	−15.2±5.38[a]
Avec irrigation des branchies ($n = 5$)	28.8±4.83[c]	19.2±1.76	+9.6±4.06[b]

[a]La valeur du flux net est significative à $P < 0.05$. Comparaison des moyennes obtenues sur les têtes irriguées et non irriguées : [b]$P < 0.01$; [c]$P < 0.001$.

Pour les branchies irriguées initialement le flux entrant de Cl^- est trois fois plus élevé que pour les branchies non irriguées ; la valeur moyenne du flux entrant mesuré sur les têtes non irriguées est trois fois plus faible ($P < 0.001$) que celle observée in vivo. Les flux sortants ne diffèrent pas statistiquement in vivo et in vitro que les branchies aient été irriguées ou non. Alors qu'une perte nette de Cl^- peut être observée sur les branchies initialement non irriguées, la valeur moyenne du flux net des branchies irriguées n'est pas statistiquement différente de 0 ($P < 0.10$). Cependant, les résultats individuels montrent qu'une seule préparation a un flux net nul alors que les quatre autres sont le siège d'une absorption nette de Cl^- d'importance variable, d'où la valeur élevée de l'erreur standard correspondante. En outre, la valeur moyenne du flux net mesuré sur les têtes initialement irriguées est du même ordre de grandeur que celle qu'on peut attendre chez les animaux in vivo, compte tenu de l'excrétion urinaire de Cl^-.

Il semble donc que l'irrigation externe des branchies avec de l'eau douce pendant l'opération empêche la détérioration des mécanismes de transferts du Cl^-. Dans les expériences antérieures sur la tête perfusée de truite, l'absence d'irrigation externe des branchies ne semblait, par contre, pas affecter les mécanismes de transferts branchiaux de Na^+ en eau douce (Payan et al. 1975). Ces observations suggèrent une relative indépendance des flux entrants branchiaux de Na^+ et Cl^- déjà proposée par de nombreux auteurs (Maetz et al. 1976). Les effets différents de l'absence d'irrigation sur les transferts de Na^+ et Cl^- pourraient s'expliquer par la localisation éventuellement différente des sites de transports actifs de ces deux ions sur les faces basale pour Na^+ (Kerstetter et al. 1970 ; Kerstetter et Keeler 1976) et apicale pour Cl^- (Kerstetter et Kirschner 1972 ; de Renzis 1975).

Une question qui reste posée est la viabilité d'une telle préparation pour des expériences de longue durée. Ce problème fait l'objet d'études en cours. Les premiers résultats obtenus dans des conditions identiques à celles décrites ici, indiquent que environ 75 min après le début de la perfusion le flux sortant devient nettement supérieur au flux entrant de Cl^-.

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Toxicity of a Mixture of Metals on Freshwater Algae

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Recommended levels of a number of metals for Great Lakes Water Quality Objectives were found to be very toxic to freshwater algae when these metals were present simultaneously in lake water and culture medium. The diatom tested was more sensitive to metal toxicity than the blue-green and green algae.

Key words: metals, Water Quality Objectives, toxicity, algae, diatom, primary productivity

WONG, P. T. S., Y. K. CHAU, AND P. L. LUXON. 1978. Toxicity of a mixture of metals on freshwater algae. J. Fish. Res. Board Can. 35: 479–481.

Nous constatons que les niveaux recommandés d'un certain nombre de métaux dans l'établissement des objectifs de qualité des eaux des Grands lacs sont très toxiques pour les algues d'eau douce quand ces métaux se trouvent simultanément dans l'eau de lac et dans le milieu de culture. La diatomée testée est plus sensible à la toxicité de ces métaux que les algues bleu-vert et vertes.

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IN 1972 the governments of Canada and the United States of America signed a Great Lakes Water Quality Agreement to develop and recommend specific water quality objectives for the waters of the Great Lakes to ensure against the loss of beneficial uses. Four years later the Water Quality Objectives Subcommittee recommended, among other things, a number of specific objectives for metals to protect the aquatic biota and the most sensitive use of waters (International Joint Commission 1976). These objectives (in micrograms per litre of water) are as follows: arsenic 50; cadmium 0.2; chromium 50; copper 5; iron 300; lead 10 for Lake Superior, 20 for Lake Huron, and 25 for the remaining lakes; mercury 0.2; nickel 25; selenium 10; and zinc 30. Concentrations are expressed as total metals, except mercury which is filtered or soluble. These objectives are generally based on the highest concentrations of metals causing no significant harmful effect to the aquatic organisms. The supporting data were obtained from published field and laboratory bioassays of lethal or sublethal toxicity with a single metal. However, in almost all natural waters, metals are present together in solution; when mixed together they have supplemental synergistic or antagonistic effects on biota (Eaton 1973; Sprague and Ramsay 1965).

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TABLE 1. Decrease of primary productivity of *Scenedesmus quadricauda* at 20°C in a mixed-metal solution using concentrations of individual metals recommended for Great Lakes Water Quality Objectives.

Concn. of mixed metal solution	Primary productivity (% ±SE)	
	CHU-10 medium	Hamilton H. water
No addition	100±5	100±6 (43)[b]
10%[a]	60±9	79±17 (34)
50%[a]	58±5	31±16 (13)
100%[a]	32±4	22±31 (9)

[a]100% metal solution contains the following metals (in micrograms metal per litre of solution): sodium arsenate (50); cadmium nitrate (0.2); potassium dichromate (50); copper sulfate (5); ferrous sulfate (300); lead nitrate (25); mercury chloride (0.2); nickel sulfate (25); sodium selenite (10); and zinc chloride (30).

[b]Numbers in parentheses are percentage with respect to CHU-10 as 100%.

Therefore, setting of water quality objectives based on experiments with single metals may not adequately protect aquatic organisms.

We examined the effects of individual metals as well as a mixture of metals at concentrations recommended as objectives on algae. Four axenic cultures of algae were chosen to represent a range of algae and diatoms in the Great Lakes waters. Two green algae, *Scenedesmus quadricauda* and *Chlorella pyrenoidosa*, a blue-green alga, *Anabaena flos-aquae*, and a diatom, *Navicula pelliculosa*, were grown in a modified CHU-10 medium (Chu 1942) at 20°C on a rotary shaker (100 rpm) under conditions of 18 h of light (5000 lx) and 6 h of darkness. Ethylenediaminetetraacetic acid was not added to the medium because we wanted to avoid an unnaturally high degree of chelation of the metals. When the cells reached the logarithmic phase of growth (about 1 wk), they were used to inoculate two test media — CHU-10 and Hamilton Harbor water. Both media had similar pH and hardness. Primary productivity and cell number were the parameters used to test the effects of metal on algae. The procedures of Malanchuk and Gruendling (1973) for the primary productivity and of Hutchinson and Stokes (1975) for cell number were employed. The metals were introduced as inorganic salts (see footnote to Table 1). The bioassays were carried out with three replicate samples. To minimize trace metal contaminations, scrupulous care was taken in cleaning glasswares and in preparing media.

From our own experiments and from the data in published reports, individual metals at the recommended concentrations are not toxic to the tested algae. However, when the metals were added together to a commonly used algal growth medium, CHU-10, the solution became very toxic to the algae. For example, it inhibited about 70% of the primary productivity of *S. quadricauda* when compared with the control (growth medium without addition of metals, Table 1). In fact, even one tenth of the concentrations decreased 40% of the primary productivity of the algae. Similar results were obtained when cell number was used as another indicator. Since CHU-10 medium represents relatively unpolluted lake water, we chose water from Hamilton Harbor in Lake Ontario as a representative for polluted and eutrophic water. With Hamilton Harbor water as the medium, the metal solution again showed inhibitory effects. Note that the lake water could support only 43% as much productivity as the laboratory medium. This is not unexpected, since Hamilton Harbor water was shown to contain low concentrations of various metals (Chau et al. 1970) which could have an additional toxic effect on the algae. The metal toxicity was more pronounced at 20°C than at 10 or 5°C. The lesser effects at the lower temperature could be due to a number of reasons, such as a decrease in metal solubility, cell metabolism, or membrane permeability to the metals.

The relative metal toxicity also varies with different algal species. With the limited species tested, the diatom was the most sensitive, followed by blue-green and green algae. A similar order of algal sensitivity to copper has been reported by Maloney and Palmer (1956).

While there is a growing quantity of information on the toxicity of individual metals to aquatic biota, not much is known about the synergistic effects when they are present together. Copper and nickel were found to act synergistically, whereas cadmium and selenium acted antagonistically on *Chlorella* (Hutchinson and Stokes 1975). Cadmium was found to inhibit the toxicity of copper to *Selanastrum* (Bartlett et al. 1974). On the other hand, cadmium was reported to act synergistically with copper and zinc in inhibiting carbon uptake by the phytoplankton in the lake (Wissmar 1972). Obviously the mechanisms involved in the multiple-metal toxicity are very complex. Regardless of the mechanisms involved, water that contains the allowable metal concentrations in the Water Quality Objectives would be detrimental to the algae. Because algae are the primary producers and are at the bottom of the food chain, their inhibition by metals would, in turn, affect the well being of the aquatic communities.

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Comment on the Distribution of Vertebral Number in North American Sculpins of the *Myoxocephalus quadricornis* Complex

Our interpretation (Fig. 1) of the data of McAllister and Aniskowicz (1976) does not entirely support their conclusion (p. 2796) that "the coastal fourhorns divide into two groups, one south of 60° and one north of 60°N," for although the lowest means occurred south of the latitude of 60° and the highest north of 60°, relatively low means occurred north of 60° (at about 70°) in the coastal area close to the Mackenzie River. Furthermore, the general configuration of the contours suggests the existence of a continuous gradation between the maximal count in the fourhorn and the minimal count in the deepwater sculpin. This would be expected, for as McPhail and Lindsey (1970, p. 321) noted, "the extreme forms seem joined by a continuum of morphological intermediates." In the detail of the figure, however, there are some interesting features which include the high count at the Ferguson River and the low counts at Sophia and Stanwell-Fletcher lakes.

The latter could result from a relatively greater coupling of the gene pools of the fourhorn and deepwater sculpins as in one of the alternate explanations McAllister gave (p. 2796) for the relatively low fourhorn counts in the southern Hudson Bay system, i.e. the injection of deepwater sculpin genes into the area about Chantrey Inlet via tributaries such as the Bach River. McAllister hypothesized (p. 2795) that the higher count at the Ferguson River occurs because movement is possible between populations there and in the sea, and this movement against the current selects for larger individuals which in turn have larger vertebral counts. Curiously, the

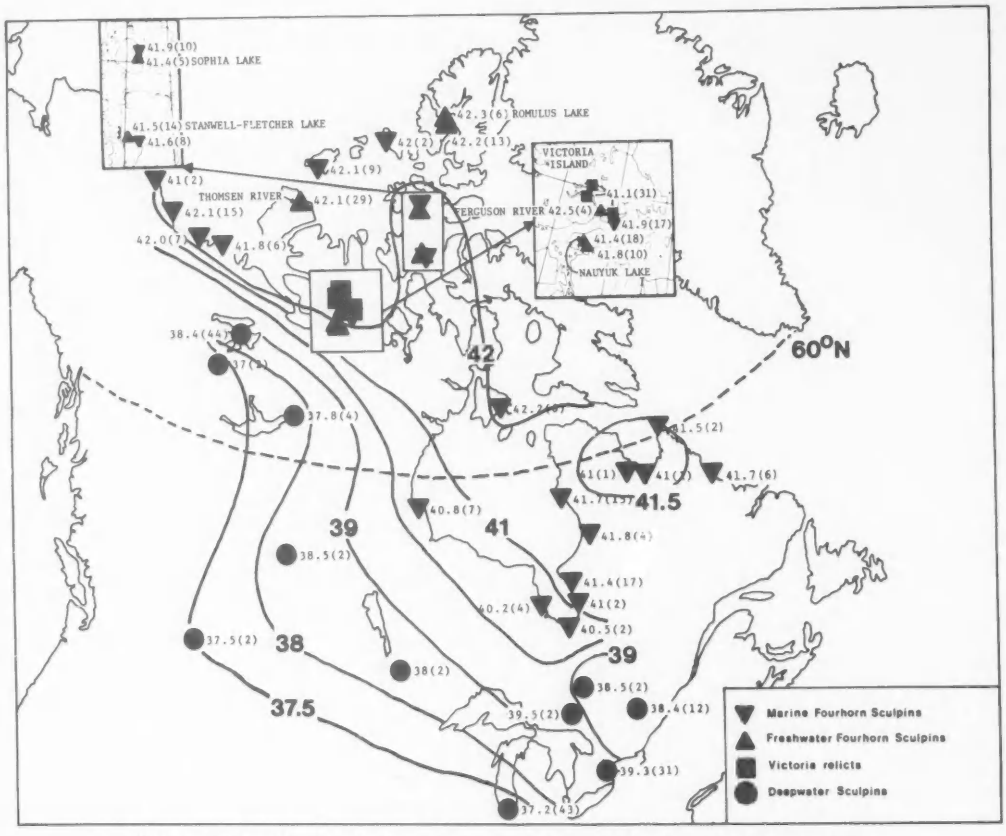

FIG. 1. Lines of equal (mean) vertebral number (from McAllister and Aniskowicz 1976).

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sculpins of the "three-lake sample," even though movement to the sea appears equally possible, were believed by McAllister (p. 2796) to "constitute gene pools distinct from the brackish–marine population" and is in their view a freshwater relict form. McAllister then proposed several methods through which the "three-lake" populations could have become established, apparently from the fourhorn.

In one they visualized the existence of a "common brackish gulf," but did not suggest how the "gulf" came about or how long it persisted. It seems now that such a region of brackish water could have had an association with a distribution of freshwater under a perennial sea ice cover speculated on by Barber and Murty (1978). From present evidence, these authors implied the existence of a perennial sea ice cover during one or more brief episodes since the most recent glaciation, under which freshwater from runoff spread with little mixing with the underlying sea water. Consequences with regard to certain animal distributions were also implied, based on a distribution of freshwater over the sea and under the ice off the coast of Alaska and into the archipelago. Should a freshwater population, i.e. of the "three-lake" population, have existed, it also could have moved over the sea under the ice and established gene flow with other fourhorn populations, assuming that interbreeding could occur (McPhail and Lindsey 1970, p. 321). Another possibility is that the deepwater sculpin may also have moved into the layer "voluntarily" in a mechanism reverse to that proposed by McAllister (1961, p. 59), i.e. it moved from river and lakes into the freshwater layer over the ocean, again interbreeding with the fourhorn. In particular, the Victoria Island relict may have originated in this way; a view that the "equivocal" nature of the evidence concerning the relationship of the relict (McAllister p. 2798) would support. — F. G. Barber, *Department of Fisheries and the Environment, Fisheries and Marine Service, Ocean and Aquatic Affairs Branch, Marine Sciences and Information Directorate, Ottawa, Ont. K1A 0E6*

Barber, F. G., and T. S. Murty. 1978. Perennial sea ice — some physical and biological consequences. SCOR/SCAR Meeting, Montreal, May, 1974. (In press)

McAllister, D. E. 1961. The origin and status of the deepwater sculpin, "*Myoxocephalus thompsonii*", a Nearctic glacial relict. Nat. Mus. Can. Bull. No. 172: 44–65.

McAllister, D. E., and J. Aniskowicz. 1976. Vertebral number in North American sculpins of the *Myoxocephalus quadricornis*-complex. J. Fish. Res. Board Can. 33: 2792–2799.

McPhail, J. D., and C. C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Bull. Fish. Res. Board Can. 173: 381 p.

Electrophoretic Distinction in Rainbow (*Salmo gairdneri*) and Cutthroat (*S. clarki*) Trout

I would like to comment on possible confusion resulting from a recent paper dealing with the electrophoretic distinction of rainbow (*Salmo gairdneri*) and cutthroat (*S. clarki*) trout (Reinitz 1977). Reinitz states that phosphoglucoisomerase (PGI) is "a reliable biochemical marker for the distinction of genetically pure samples of the two species." This is not true; populations of cutthroat trout from the Pacific Ocean coastal drainages of western North America have the same common PGI phenotype as rainbow trout (Allendorf and Utter 1977). In addition, genetic variation for PGI in both species further reduces the value of this locus as a clearcut marker distinguishing these species (Allendorf 1975 and unpublished data).

The difference described by Reinitz at the *PGI-3* locus is valid for the particular stocks of cutthroat and rainbow trout he examined. My comments here, therefore, do not affect the major conclusion of the article; this marker (*PGI-3*) can be used to characterize the native cutthroat trout found in the mountainous regions of western Montana. It should be recognized, however, that the extension of this observation to comparisons of these two species in general is not valid. — Fred W. Allendorf, *Department of Zoology, University of Montana, Missoula, Mont. 59812, USA*

Allendorf, F. W. 1975. Genetic variability in a species possessing extensive gene duplication: Genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. Ph.D. Thesis. University of Washington, Seattle, Wash.

Allendorf, F. W., and F. M. Utter. 1977. Population genetics of fish. *In* Fish Physiology, Vol. 8. Academic Press, Inc., New York, N.Y.

Reinitz, G. L. 1977. Electrophoretic distinction of rainbow trout (*Salmo gairdneri*), west-slope cutthroat trout (*S. clarki*), and their hybrids. J. Fish. Res. Board Can. 34: 1236–1239.

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Environmental Toxicity of Aquatic Radionuclides. Models and Mechanisms. *Edited by Morton W. Miller and J. Newell Stannard. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich., 1976. xv + 333 p. $26.50.*

This book is a welcome addition to a field where most of the literature consists of classified, or limited-circulation material poorly known outside of a small circle of radiochemists. It is worth the price for its reference lists alone.

Section I, on the biogeochemistry of transuranic elements, contains papers by many of the most eminent scientists in this field. Many important results and ideas are presented.

There are, however, some areas that could be improved. There are few background ecological or chemical data in any of the papers. Without such information, the unwary reader will be tempted to conclude that transuranics behave similarly in all aquatic and sediment systems, an assumption that is dangerous (see, for example, the paper by Jinks and Wrenn later in the volume). Heatherington (p. 124) claims that large transuranic discharges at Windscale have no effect on the Irish Sea's flora and fauna, while Miettinen (p. 29) calls plutonium one of the most toxic of elements known. Neither gives any evidence to back up these claims. While plutonium may be one of the more toxic elements, many other elements and compounds are more abundant and immediately toxic, for example, formaldehyde, cyanide, cadmium, methylmercury, and trimethyl arsine. There is no indication in some of the papers of how large are errors in measurement. Many papers are curiously deficient in presentation of methods and it is difficult to assess how comparable the results of different studies are. For example, Livingstone and Bowen present data for americium 241, which is difficult to measure well, with scarcely a mention of the methods employed. Their theory of migration of americium relative to plutonium in sediments is interesting, but unconvincing due to a somewhat chaotic presentation. The above are minor shortcomings in an interesting section.

Section II, on modeling and measurements, is less impressive. The papers offer almost nothing new. Systems models are not substantially different from those used for other environmental problems and no more convincing. The summary of desirable measurements is naive and out of date, done better in many other works. The paper on colloidally bound isotopes totally ignores the huge body of literature in soil science and limnology on colloidal binding of nonradioactive elements, where many useful comparisons might be made.

The model testing (experimental?) section revives one's interest. Eyman et al.'s detailed description of methods employing short-lived gamma emitting plutonium isotopes to ease the difficult problems of radiochemical separations, alpha counting, and long-term contamination will excite the ecological experimentalist to want to adopt the methods (at least until he discovers the cost of these isotopes).

The Hudson Estuary study by Jinks and Wrenn offers an interesting account of the dramatic changes in cesium behavior in a region of changing salinity. It is obvious that the chemistry of cesium in estuaries is much more complicated than in either marine or freshwater systems and must be studied in much more detail. An input–output mass balance summary would have added substantially to the value of the paper.

The following paper, by Wrenn and Jinks, should be read by every alarmist in the country. Perhaps antinuclear activists should shift their concern from plutonium to naturally occurring radium and uranium, concentrated by mining activities which appear to present enormous problems compared to transuranic elements. One would like to see estimates of radium and uranium released from fossil fuel mining and processing and from geothermal sources, just to put the radio nuclide problems associated with the nuclear industry per se in perspective.

In the last section, on general ecological studies, the paper by Emery and Klopfer suffers once again from mass balance considerations — they simply do not know inputs well, though this is not their fault. As long as ecological studies are mere appendages to nuclear engineering, it will be impossible to eliminate such shortcomings, and there is a critical lack of ecosystem studies of transuranic elements where inputs are well known.

The final paper, by Templeton, contains useful references, but it is far too short.

Two areas are totally uncovered by the volume. There is little information on hydrological radionuclide problems, or on the properties of radionuclides in terrestrial soils. Both have a critical bearing on the aquatic environment. Three references given at the end of this review give some coverage of these topics.

A number of minor typographical and editorial errors also mar the book.

Finally, one is disappointed to find that the book ends without a synthesis paper or summary statement by the field's luminaries. Instead, there is a rather rambling panel discussion.

Canadian readers will also be struck by the fact that there was not a single Canadian scientist participating in the symposium. Even if the budding nuclear industry in this country were not important enough to warrent ecological concern, we must know the ecological effects of transuranic elements and other radionuclides discharged by the U.S. into the Great Lakes and coastal waters, and by the transboundary movement of fallout from nuclear bombs. One hopes that this will be the first of many informative summaries on radionuclides in the environment. Perhaps in the next one, some courageous author will tackle the thorny environmental issues of long-term waste disposal and fuel reprocessing, with which many governments are wrestling (see references below).

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EICHOLZ, G. F. 1976. Environmental aspects of nuclear power. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich. 683 p.

IAEA. 1976. Transuranium nuclides in the environment. IAEA (Int. Atom. Energy Agency). 724 p.

OCEANUS. 1977. (complete issue). Oceanus 20(1).

The Biology of Diatoms. *Edited by D. Werner. Botanical Monographs, Vol. 13. University of California Press, Berkeley, Calif., 1977. vii + 498 p. $32.50.*

This book includes contributions on all major aspects of diatom biology from some of the world's leading experts on this large group of plants. Coverage of the recent literature (prior to 1974 or 1975) seems sufficiently thorough that most omissions of particular subjects may be taken as indicating areas where research on diatoms is thin or lacking. However, one wonders whether there was not sufficient information for a short chapter on the evolutionary history of diatoms as reflected in the fossil record. Generally, organization is adequate to excellent. The writing, particularly by contributors whose mother tongue is not English, is somewhat uneven. Typographical errors, however, are few.

Before going further, I should point out that my primary interest is in algal physiology and its application to ecology, and that I am likely to be more critical of these areas than of those I am less familiar with. Also, as one interested in particular aspects of the biology of all algae rather than all aspects of a particular group, I find this sort of book (devoted to one group) somewhat frustrating to read. I am sure the contributors must have often felt the same frustration in trying to restrict their discussion of a particular phenomenon to work on diatoms when the same phenomenon may be much better illustrated in work on another group of algae. Often reference to work on other algal groups would have been helpful but frequent digressions would have distracted from the principal intent of the book, and only rarely have contributors done this. However, in its main purpose as a storehouse of present knowledge of diatom biology, this book leaves little room for criticism.

A major part (155 p.) is devoted to five chapters on various aspects of physiology. I found those on silicon metabolism, heterotrophic nutrition, and biochemical composition to give well-organized and thorough coverage of these topics, reflecting the recent high level of activity in these areas. The chapter on photosynthesis, however, I found rather disappointing. This was in part due to the rather limited literature on which the author could draw. Few advances in photosynthesis have depended on work involving diatoms and even papers confirming for diatoms results found elsewhere are rare, a surprising fact in view of the major role of diatoms in oceanic and world photosynthesis. However, the author doesn't make full use of what is available. For instance, he does not have to assume that the pathways of CO_2 fixation are similar in diatoms to those in other algae (Coombs and Volcani 1968). And while the effect of light and temperature on photosynthesis are examined in detail, why are the several observations available in the literature on the effects of nutrient deficiency on photosynthesis of diatoms largely ignored? It is also surprising that the ideas on light and temperature adaptation of photosynthesis in algae presented here have not appreciably progressed beyond the rather simple-minded ones put forward by Steeman Nielsen over 15 years ago. While these may be true as far as they go, they are far short of even a satisfying explanation. Nothing is made of this deficiency.

An attempt has been made to round up the remaining aspects of physiology in a chapter on the growth and culture of diatoms. The result is a rather diffuse discussion, not at all helped by the artificial separation of results from batch and continuous cultures and further weakened by the addition at the end of the chapter of brief notes on more recent papers, apparently in an attempt to update the chapter. One must search the index for information on the effects of toxicants on diatoms. More information is available on heavy metals than is mentioned in passing in the ecology chapters. This chapter would also have been the place to point out specific areas where experimental work on diatoms is lacking.

Two chapters (on sexuality and motility) could be grouped as behavior (58 p.). While my limited knowledge in these areas prevents critical examination, both treatments seemed complete with differing viewpoints presented clearly and fairly. However, both could have been improved by more thorough definition of terms unique to these fields.

A sizeable (50 p.) chapter is devoted to a thorough discussion of ultrastructure, illustrating the great advances made in this area during the past 20 years. The description of methods is perhaps too detailed for the general reader. While the discussion is excellent, the chapter suffers severely from the scarcity of illustrations. Only three electron micrographs are given in the chapter. One wonders why eight electron micrographs of shell structure were included without adequate discussion in the introductory notes on taxonomy when the same expense would have been so much more useful in illustrating the discussion of ultrastructure. Failing that, some simple line drawings would have been a great help in guiding the reader not familiar with recent advances in ultrastructure research.

The final three chapters (180 p.) are devoted to ecology. Parts of all three treat floristic surveys; the rest of each chapter considers processes and interactions in communities containing diatoms. The discussion of freshwater ecology emphasizes observational correlations — what species are found under what conditions. The discussion implies to a distressing degree that species prefer the conditions under which they are found rather than occur where biological processes of grazing and competition allow them. The discussion is also flawed by unfounded and vague statements and tends to give the idea that more is known than really is. At any rate, this chapter illustrates the degree to which observational ecology has outflanked the more experimental approaches it needs for confirmation.

While most of the book is a "meat and potatoes" course of information on diatoms, the latter half of the chapter on marine planktonic ecology was for me dessert. This controversial advocacy of biological factors (particularly grazing) as opposed to physical and chemical factors in species succession had me alternately cheering and growling. While the arguments presented do a great service in drawing attention to the biological environment of diatoms (and other planktonic algae), the importance of the chemical and physical environment is underestimated. Recent work on eutrophication is an example of the great effect an altered chemical environment can have on the species composition of algae.

There are other points I would take issue with. The argument developed in the treatment of marine planktonic ecology that half-saturation values of nutrient uptake fail to predict species succession in nature does not take account of the fact that a combination of maximum rates and half-saturation values is required to reflect rates of uptake at low concentrations. Nor does it recognize that differences both in rates of uptake and in yields per unit of nutrient taken up are important in determining the outcome of competition. Elsewhere in the book, the mixing of half-saturation values and maximum rates for both short-term nutrient uptake and growth in Table 2.4 is misleading in light of theoretical arguments that these can be expected to differ and the evidence that, for at least some nutrients, they do differ greatly.

This book will be useful to any reader, student or researcher,

with a general interest in diatoms. Even someone actively engaged in research on some aspect of diatoms can expect to find useful summaries of areas other than his own. While the book generally fails to advance new ways of looking at diatom biology, it is rich in information. It is a must for libraries aiming for completeness in the aquatic sciences or phycology, and should be seriously considered by anyone concerned with diatoms.

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COOMBS, J., AND B. E. VOLCANI. 1968. Studies in the biochemistry and fine structure of silica shell formation in diatoms: silicon-induced metabolic transients in *Navicula pelliculosa* (Breb.) Hilse. Planta 80: 264–279.

New Strategic Factors in the North Atlantic. *Edited by Christoph Bertram and Johan J. Holst. Universitetsforlaget/IPC Science and Technology Press, Oslo and Guildford, 1977. 193 p. $25.00.*

This book contains 13 essays commissioned by the International Institute for Strategic Studies and the Norwegian Institute of International Affairs, both unofficial bodies claiming to express no opinions of their own.

The first four chapters make plain the remarkable increase in the last 20 years of the strength of the Soviet Union Navy and of its deployment in the North Atlantic, and show how this strength, together with new naval technologies, could greatly hamper United States intervention in any military action in Europe in which Russia was involved.

A short chapter by N. Ørvik of Queens University, concerns Canadian defence and how this may be influenced by United States concern for security to the north and the need for western nations to keep open the Atlantic approaches to Europe, quite apart from Canada's need to protect its sea lanes, fishing zones, and offshore installations.

These concerns require a broad view of the situation, which the book provides. These essays should be helpful and interesting to those concerned with our national security, and to the student of foreign affairs, but there is nothing here of direct interest to the marine scientist.

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The Political Implications of North Sea Oil and Gas. *Edited by Martin Saeter and Ian Smart. Universitetsforlaget/IPC Science and Technology Press Ltd., Oslo and Guildford, 1975. 168 p.*

This paperback reports presentations to a conference in Tønsberg, Norway, in 1975, which was sponsored by the Norwegian Institute of International Affairs, Oslo, and the Royal Institute of International Affairs, London, both unofficial bodies claiming to express no opinions of their own.

The seven chapters are authoritative and serious in tone, and make it obvious that the five nations with oil and gas reserves in the North Sea must achieve many adjustments among themselves in international law, in trade, and in the financing of staggeringly expensive operations, as well as looking to their relationships with the Soviet Union. It is clear that there are ahead, many years of bargaining and negotiation.

The book is better at laying out the problems than in suggesting solutions, although the chapter on finance by E. Brown does offer some ideas on how governments may assist industry and secure equitable tax revenues.

This collection should be of interest to politicians and economists, but, apart from some passing references to fishing rights and to pollution, there is nothing of specific interest to the marine scientist.

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Navigation Primer for Fishermen. *Capt. F. S. Howell M.B.E. Fishing News Books Ltd., Farnham, Surrey, England, 1977. vx + 182 p. £5.75.*

It is common for working British fishermen who seek to qualify for certificates of seamanship to take some weeks or months ashore for training and the necessary written and oral examinations. While doing so they are not earning and courses of instruction are necessarily reduced to basics and are kept as short as possible.

This is shown in this book, which is intended for men with some 4 years experience at sea who want to acquire a certificate as second hand. It covers the compass, charts and chartwork, basic navigation and nautical astronomy, and the principles of ship stability. Explanation has been reduced to the minimum, being mostly replaced by definitions to be learnt by rote. There is a sprinkling of mnemonics, usually for avoidance of ambiguity in applying corrections. Calculations are never given in algebraic form but as plain and realistic worked examples. The exposition throughout is clear and unambiguous and it is conceivable that the student could learn from the book by himself what he needs to know. Used as a primer, with an experienced instructor to amplify and further illustrate it, it will be excellent.

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Biological Control of Water Pollution. *Edited by Joachim Tourbier and Robert W. Pierson Jr. University of Pennsylvania Press, Philadelphia, Pa., 1976. ix + 340 p. $20.00.*

This book would be more appropriately named "Biological Alternatives in Wastewater Treatment" since its intent is to consider the use of plants and animals in purifying domestic and industrial effluents. The book is a result of the Interna-

tional Conference on Biological Water Quality Improvement Techniques held at the University of Pennsylvania in March 1975. The conference organizers "... personally wanted to gain more knowledge about biological wastewater alternatives and believed that scientists and decision makers in academic, government, and industrial communities would also be very interested in these techniques ..." (p. 1).

Experts from seven countries contributed to the book. Canada was not represented. Perhaps little or no work is being done in Canada or our northern latitude/cold winter climate precludes efficient use of the biological systems discussed.

The book has a brief introduction and is divided into six sections containing a total of 39 papers. The editors are to be commended on keeping the papers short (the average length is about 10 pages) and to the point. The diversity of contributors (city planners, politicians, scientists, administrators, medical doctors, economists, lawyers, engineers, etc.) almost dooms the book but, on the strength of several excellent key papers, the editors succeed in drawing together the presentations in reasonably complementary fashion. However, the book does suffer from many papers of dubious quality and relevance. Conference organizers should make more use of anonymous refereeing, publishing only those papers that survive the process.

The introduction is by E. W. Furia, an attorney and city planner. These credentials notwithstanding, he sets up the reader for what is to follow and makes the points that "... biological systems — no matter how effective — can never be a substitute for designing industrial processes which prevent pollutants from entering streams and rivers in the first place." (p. 3); and "... the fact that biological systems are inexpensive compared to conventional systems means they will probably present fewer profit opportunities for treatment plant designers. This is unfortunate, but realistically this will also delay implementation of the system." (p. 4). But, in our society, since when did lower production costs have to mean lower profits?

Part I, "A Rationale for the Biological Approach," contains five papers and sets the background (mainly social–political–economic) for discussing biological alternatives in the treatment of wastewaters. Contributors include E. Muskie and W. D. Ruckelshaus but I. McHarg's irreverant lead-off article (Biological Alternatives to Water Pollution) is the most entertaining. To wit: "The implicit assumption, the inalienable right governing water management, is that thou shalt defecate into thy neighbor's drinking water and, further, if for any reasons of delicacy, hygiene, or prudence you prefer not to do so, be assured that thy municipality shall do it for thee." (p. 7). He makes the reasonable suggestion that administrative structures within the U.S. Water Pollution Control Act should correspond with watersheds and physiographic regions rather than with arbitrary political subdivisions. Muskie's contribution (The Economy, Energy, and Clean Water Legislation) lays out political strategies designed to increase public support of environmental objectives. Included is the pitch that environmental controls will have a stimulative rather than depressive economic impact in the U.S., a view that is now being seriously questioned. He also raises the point that "Phosphorus, nitrogen, and potassium have been the targets of water pollution clean-up efforts for years, and then viewed as a sludge disposal problem, while the cost of fertilizer has escalated to unacceptable levels." (p. 17). The only non-U.S.-oriented article in Part I is by T. A. Lambo, World Health Organization, Geneva (The Protection and Improvement of the World's Drinking Water Quality) which contains a brief but intriguing account of ancient water purification practices.

Part II, "Limnological Overview," consists of three papers intended to provide scientific basics for the papers that appear in the following two parts. R. Patrick's two page "Overview of Aquatic Ecosystems" is so general that it is vague and of limited use to the book. The most pertinent part of her second paper (The Role of Aquatic Plants in Aquatic Ecosystems) is on the effects of trace and heavy metals on plant growth and the accumulation of heavy metals by plants (2 pages out of 6). T. Blott's contribution (Nutrient Cycles in Natural Systems: Microbial Involvement) is basic limnology and contains, in the summary, a few pertinent paragraphs about man's influence on nutrient cycles. Nothing is said about invertebrates or fishes in Part II. Exclusion of this section would not have seriously impaired the message of the book.

The same can be said of Part III: "Drinking Water Problems." Two of the five papers deal with carcinogens in drinking water and are highly repetitious. Judicious editing could have avoided this. (However, the paper by S. S. Epstein – Potential Carcinogenic Hazards due to Contaminated Drinking Water – is a scholarly treatment.) The remaining papers represent diverse topics, indicating how heterogeneous this section is. Only the paper by S. Al Azharia Jahn (Sudanese Native Methods for the Purification of Nile Water during the Flood Season) approaches relevance to the theme of the book.

Parts IV (Biological Treatment of Wastewater – 16 papers) and V (Biological Treatment and Aquifer Recharge – 5 papers) are the heart of the book. Papers by K. Seidel (Macrophytes and Water Pollution), C. D. McNabb Jr. (The Potential of Submersed Vascular Plants for Reclamation of Wastewater in Temperate Zone Ponds), J. de Jong (The Purification of Wastewater with the Aid of Rush or Reed Ponds), B. C. Wolverton et al. (Application of Vascular Aquatic Plants for Pollution Removal, Energy and Food Production in a Biological System), and F. Spangler et al. (Experimental Use of Emergent Vegetation for the Biological Treatment of Municipal Wastewater in Wisconsin) are excellent and adhere to the theme of the book. The paper by N. M. Trieff et al. (Sewage Treatment by Controlled Eutrophication Using Algae and *Artemia*) should be grouped with these other "artificial" systems for treating wastewater. These six contributions detail the often spectacular elimination of inorganic and organic wastes from water. For example, in Seidel's paper:

1. *Mentha aquatica* reduced *E. coli*, Enteroccocci, and *Salmonella* 90% after 2 h contact (Table 14-3). 2. Short-term pH neutralization by macrophytes ($4.0 \rightarrow 6.5$; $12.0 \rightarrow 6.7$) during a morning or evening (Table 14-4). 3. Sludge water quality improvement using beds of *Phragmites*: transparency (cm), $0 \rightarrow 53.0$; BOD_5 (mg/L), $1300 \rightarrow 5.5$; settling solids (cm^3/L), $825 \rightarrow 0$; (from influent sludge water to discharge water). Seidel's paper also includes explanations of the mechanisms of purification and this was gratifying.

In Wolverton et al.: One acre (0.40 ha) of *Eichhornia crassipes* can potentially remove >1591 kg of N and >364 kg of K annually from sewage effluent, absorb and metabolize >68 kg of phenol every 72 h, and remove >120 g of trace heavy metal contaminants in 24 h. The same paper deals with the conversion of harvested vascular aquatic plants to useful products such as methane gas, fertilizer, feeds for poultry and livestock, etc.

Papers by P. G. Hunt and C. R. Lee (Land Treatment of Wastewater by Overland Flow for Improved Water Quality) and D. F. Whigham and R. L. Simpson (The Potential Use of Freshwater Tidal Marshes in the Management of Water Quality in the Delaware River) emphasize the use of "natural ecosystems to process wastewaters." Judging from recent literature (e.g. Woodwell 1977, and see recent numbers of *Water Research*), these methods are becoming increasingly

more accepted alternative wastewater treatments.

A group of three papers (J. C. Goldman and J. H. Ryther – Waste Reclamation in an Integrated Food Chain System; R. L. Carpenter et al. – Aquaculture as an Alternative Wastewater Treatment System; and R. Dinges – A Proposed Integrated Biological Wastewater Treatment System) use a food-chain, mariculture/aquaculture approach. However, it is hard to imagine wide acceptance of food produced from domestic sewage by a society that insists on using unblemished tomatoes to make tomato paste (Pimental et al. 1977). Nevertheless, the contribution by Carpenter et al. is particularly good. In the six cells of the sewage lagoons used, the secondary treatment standard for BOD_5 (30 mg/L) was reached by the second cell (the raw water BOD_5 was 184 mg/L) and fell to 6 mg/L by the sixth cell. Secondary standards for suspended solids (30 mg/L) and fecal coliform (200/100 mL) were reached in the effluent of the fifth cell. Overall, nitrogen was reduced 86% and phosphorus was reduced 77%.

Significantly higher quality effluent resulted when fish were present in the system. Suspended solids criteria were not met without them whereas BOD_5 and fecal coliform were. *Tilapia nilotica* increased from an initial 4 lb (175 individuals) to 163 lb (2339 individuals) in 4 mo. Channel catfish biomass increased from 600 lb to an estimated 4400 lb and an initial 85 lb of golden shiner minnows produced an estimated 535 lb. The remaining five papers in Part IV either discussed more conventional wastewater technologies (papers no. 27–29) or were irrelevant (papers no. 21, 22).

Groundwater and groundwater recharge was the common thread in Part V. W. E. Sopper's article (Renovation of Municipal Wastewater for Groundwater Recharge by the Living Filter Method) which deals with crop and forest responses to sewage application is the only one relevant to the book and should have been grouped with the papers by Hunt and Lee and Whigham and Simpson in Part IV.

Part VI (Implementation of Alternatives) deals mainly with the failure (mostly U.S.) to implement the innovative processes previously discussed and extends some of the considerations in Part I on this point. This section would be more correctly entitled "Impediments to Implementing Alternatives." The paper by W. M. Eichbaum (Legal and Political Restraints to Implementation of Novel Systems) is the best in Part VI and a highlight of the book although A. V. Kneese's offering (Implementation of Water Quality Plans) in which he details water management by Genossenschaften in the Ruhr is also a gem. For example, the Emscher River has been sacrificed and "... is in effect a [concrete-] lined open sewer used exclusively for waste dilution, degradation and carriage" (p. 326). To my knowledge, the concept of entirely sacrificing one river system to assure higher water quality in the others is not now knowingly being practised in North America. Eichbaum's contribution answered the recurring and obvious question I had throughout the book: If these biological alternatives are so good, why are they not being used extensively in North America? Examples of the answers are:

1. "... most pollution control agencies do not have available an adequate number of personnel with the level of sophisticated training that would permit evaluation and understanding of the concepts and opportunities inherent in an innovative pollution control technology" (p. 320); 2. "Undoubtedly, in almost all cases the application of known technology will prevail over that of novel technology in a cost–benefit analysis and this is a serious barrier to the implementation of the novel technology" (p. 321); 3. "Historically, local government has neither been in the business of being in business, which is essentially what resource recovery is, nor has it been involved with the applications of highly complex and novel technology. The risks for elected officials as they become involved in such financially and technically complex ventures are dramatic and severe. They are therefore reluctant to do so and as a result the technology does not get applied" (p. 321–322).

The book is soft-cover bound in an 8×10 in double-column format. The binding did not break throughout my vigorous reading. I found only a few typos. In fact, my only complaint about reproduction of the book is the poor quality of some of the photos. At $20, it is good value considering its contents. The six-page index appears to be comprehensive.

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(R175)

PIMENTAL, D., E. C. TERHUNE, W. DRITSCHILO, D. GALLAHAN, N. KRINNER, D. NAFUS, R. PETERSON, N. ZARECH, J. MISITI, AND O. HABER-SCHAIM. 1977. Pesticides, insects in foods, and cosmetic standards. Bioscience 27: 178–185.

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Books Received/Livres reçus

November 1977

Biology of Benthic Organisms. Proceedings of the 11th European Symposium on Marine Biology, Galway, October, 1976. *Edited by B. F. Keegan, P. O. Ceidigh, and P. J. S. Boaden. Pergamon Press, Oxford, England, 1977. xxxiii + 630 p. $50.00.* (Sixty-three papers from the Symposium on various aspects of biology of benthic organisms, primarily marine species.)

Salmon Fisheries of Scotland. *Edited by J. R. W. Stansfeld for the Association of Scottish District Salmon Fishery Boards. Fishing News Books Ltd., Farnham, Surrey, England, 1977. 79 p. $2.90.* (A small, soft-cover pocket reference guide for those responsible for salmon fishery management. Not particularly applicable to North American salmon fisheries.)

Fundy Tidal Power and the Environment. Proceedings of a Workshop on the implications of Fundy Tidal Power held at Wolfville, Nova Scotia, November 4–5, 1976. *The Acadia University Institute, Wolfville, N.S. 1977. v + 304 p. $10.00.* (The subtitle is apt.)

Disease Diagnosis and Control in North American Marine Aquaculture. Developments in Aquaculture and Fisheries Science, 6. *Edited by Carl J. Sindermann. Elsevier Scientific Publishing Co., Amsterdam, New York, 1977. xii + 330 p. $39.95.* (This volume appears to be basically a compendium on various diseases and diagnostic methods, organized in the form of a practical manual.)

The Rand-McNally Atlas of the Oceans. *Edited by Martyn Bramwell and consultants. Mitchell Beazley Publishers Ltd., London, (USA, Rand McNally and Company, Chicago, Ill.), 1977. 208 p. (1000 full color plates). $29.95.* (This is a magnificently illustrated atlas aimed primarily at the intelligent, curious public. It has a distinguished list of contributing authors, and appears to be quite up-to-date.)

Books Received/Livres reçus

December 1977

Discover the Atlantic Ocean. An exciting coloring book of fish and shellfish. *Text by George J. Flick, illustrations by Ron Clayton, and design by Janice Ford. Virginia Polytechnic Institute and State University, Blacksburg, Va., 1976. 38 p. $1.50.* (A novel and well-executed means of teaching the young about some of the inhabitants of the Atlantic Ocean. The only thing missing is some sort of key to the color patterns of the specimens; such a key would greatly enhance the book's value.)

L'Europe et les ressources de la mer : ou le débat sur la croissance économique. *Orio Giarini, Henri Loubergé et Henri Schwamm.* Editions Georgi. St-Saphorin (Suisse), 1977. 173 p. (To be reviewed.)

Decompression Sickness. Volume 1. The biophysical basis of prevention and treatment. *Brian A. Hills. John Wiley and Sons, New York, N.Y., 1977. x + 322 p. $31.50.* (The major purpose of the book is to explain the prevention and treatment of decompression sickness on a physiological and physical basis rather than in terms of empirical procedures.)

Fire 1. A computer program for the computation of fishery statistics on samples with aged and non-aged subsamples. *IBM 360/65, Fortran IV, G level. Nebraska Technical Series No. 1. Larry Hesse. Nebraska Game and Parks Commission, Lincoln, Nebr., 1977. 60 p.* (If future items in this series are as well prepared as this one, readers are urged to place themselves on the mailing list.)

A Guide to the Jellyfish of Canadian Atlantic Waters. *Natural History Series No. 5. C. T. Shih. National Museum of Natural Sciences, National Museums of Canada, Ottawa, 1977. 92 p.* (A beautifully illustrated, spiral-bound guide, obviously designed to last. It is also available in French.)

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Symposium on Recovery Potential of Oiled Marine Northern Environments

Editor's foreword/Note du rédacteur **J. C. Stevenson**

In Memoriam — Dr Max Blumer, organic geochemist and environmental scientist, 1923–77 **J. W. Farrington**

Introduction to the Symposium on "Recovery Potential of Oiled Marine Northern Environments" **J. H. Vandermeulen**

Session I. Long-Term Fate of Petroleum Hydrocarbons After Spills — Compositional Changes and Microbial Degradation

Analyses of aromatic hydrocarbons in intertidal sediments resulting from two spills of No. 2 fuel oil in Buzzards Bay, Massachusetts **J. M. Teal, K. Burns, and J. Farrington**

Long-term fate of a heavy fuel oil in a spill-contaminated British Columbia coastal bay **W. J. Cretney, C. S. Wong, D. R. Green, and C. A. Dawden**

Oil spill residues in Chedabucto Bay, Nova Scotia, in 1976 **P. D. Keizer, T. P. Ahern, J. Dale, and J. H. Vandermeulen**

Contribution of chronic petroleum inputs to Narragansett Bay and Rhode Island Sound sediments **E. S. Van Vleet and J. G. Quinn**

Preliminary assessment of the presence of oil in the ecosystem at Ekofisk following the blowout, April 22–30, 1977 **P. R. Mackie, R. Hardy, and K. J. Whittle**

Weathering characteristics of petroleum hydrocarbons deposited in fine clay marine sediments, Searsport, Maine **D. W. Mayo, D. S. Page, J. Cooley, E. Sorenson, E. S. Gilfillan, and S. A. Hanson**

Rates of mechanical dispersal of oil stranded in the littoral zone **E. H. Owens**

Microbial ecology studies of the *Metula* spill in the Straits of Magellan **R. R. Colwell, A. L. Mills, J. D. Walker, P. Garcia-Tello, and V. Campos-P.**

Distribution and abundance of hydrocarbon-utilizing bacteria in sediments of Chedabucto Bay, Nova Scotia, in 1976 **J. E. Stewart and L. J. Marks**

Prudhoe crude oil in arctic marine ice, water, and sediment ecosystems: degradation and interactions with microbial and benthic communities **R. M. Atlas, A. Horowitz, and M. Busdosh**

Importance of sediment working by the deposit-feeding polychaete *Arenicola marina* on the weathering rate of sediment-bound oil **D. C. Gordon Jr., J. Dale, and P. D. Keizer**

Session I. Summary and Overview: Long-term fate of petroleum hydrocarbons after spills — compositional changes and microbial degradation **J. N. Butler and E. M. Levy**

Session II. Physiological Stresses and Response in Chronically Oiled Organisms

Uptake of hydrocarbons from marine sediments contaminated with Prudhoe Bay crude oil: influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons **G. Roesijadi, J. W. Anderson, and J. W. Blaylock**

Aromatic hydrocarbons in benthic organisms from coastal areas polluted by Iranian crude oil **O. Grahl-Nielsen, J. T. Staveland, and S. Wilhelmsen**

Preliminary ecological and genetic comparisons between unperturbed and oil-impacted *Urosalpinx cinerea* (Prosobranchia: Gastropoda) populations: Nobska Point (Woods Hole) and Wild Harbor (West Falmouth), Massachusetts **T. J. Cole**

Alterations in growth and physiology in chronically oiled *Mya arenaria* from Chedabucto Bay, Nova Scotia: *Arrow* Bunker C 1970–76 **E. S. Gilfillan and J. H. Vandermeulen**

Effects of uptake and discharge of petroleum hydrocarbons on the respiration of the soft-shell clam, *Mya arenaria* **D. M. Stainken**

Absence of aryl hydrocarbon hydroxylase (AHH) in three marine bivalves **J. H. Vandermeulen and W. R. Penrose**

Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax* **C. T. Krebs and K. A. Burns**

Effects of chronic exposure to petroleum upon the growth and molting of juveniles of the arctic marine isopod crustacean *Mesidotea entomon* **J. A. Percy**

Pathological changes in a marine fish after a 6-month exposure to petroleum **J. F. Payne, J. W. Kiceniuk, W. R. Squires, and G. L. Fletcher**

Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: implications for recovery in the Wild Harbor marsh **J. J. Stegeman**
Oiled seabird rescue and conservation **R. B. Clark**
Session II. Summary and Overview: Physiological stresses and response in chronically oiled organisms **J. W. Anderson and D. C. Malins**

Session III. Long-Term Effects of Oil Spills on Marine Intertidal Communities

Recolonization of rocky shores in Cornwall after use of toxic dispersants to clean up the *Torrey Canyon* spill **A. J. Southward and E. C. Southward**
Comparison of oiled and unoiled intertidal communities in Chedabucto Bay, Nova Scotia **M. L. H. Thomas**
Florida oil spill impact on the Buzzards Bay benthic fauna: West Falmouth **H. L. Sanders**
No. 2 fuel oil spill in Bourne, Massachusetts: Immediate assessment of effects on marine invertebrates and a 3-year study of growth and recovery of a salt marsh **G. R. Hampson and E. T. Moul**
Long-term effects of an oil spill on *Fucus* macrofauna in a small Baltic bay **M. Notini**
Observations of a cold-water intertidal community after 5 years of a low-level, persistent oil spill from the *General M. C. Meigs* **R. C. Clark Jr., B. G. Patten, and E. E. DeNike**
Recovery of benthic macrofauna from chronic pollution in the sea area off a refinery plant, Southwest Finland **E. J. Leppäkoski and L. S. Lindström**
Recruitment of benthic animals as a function of petroleum hydrocarbon concentrations in the sediment **J. W. Anderson, R. G. Riley, and R. M. Bean**
Session III. Summary and Overview: Long-term effects of oil spills on marine intertidal communities **K. H. Mann and R. B. Clark**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n°1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2458. WILDISH, D. J., H. AKAGI, AND N. J. POOLE. 1977. Avoidance by herring of dissolved components in pulp mill effluents. Bull. Environ. Contam. Toxicol. 18: 521–525.

2459. SHUM, G. T. C., H. C. FREEMAN, AND J. F. UTHE. 1976. Flameless atomic absorption spectrophotometry of selenium in fish and food products. J. Assoc. Off. Anal. Chem. 60: 1010–1014.

2460. SMITH, T. G. 1977. The occurrence of a narwhal (*Monodon monoceros*) in Prince Albert Sound, western Victoria Island, Northwest Territories. Can. Field Nat. 91: p. 299.

2461. SMITH, T. G., AND J. MEMOGANA. 1977. Disorientation in ringed and bearded seals. Can. Field Nat. 91: 181–182.

2462. SMITH, T. G. 1977. The Wolffish, cf. *Anarhichas denticulatus*, new to the Amundsen Gulf area, Northwest Territories, and a probable prey of the Ringed Seal. Can. Field Nat. 91: p. 288.

2463. MAUL, G. E., AND D. E. SERGEANT. 1977. New Cetacean records from Madeira. Bocagiana 43: 1–8.

2464. VARGA, S., R. E. DOBSON, AND R. EARLE. 1977. Comparison of the elevated temperature plate count (ETPC) and MPN procedure to estimate the densities of fecal coliforms and *Escherichia coli* in soft-shell clams. J. Food Protect. 40: 763–764.

2465. ANTIA, N. J. 1977. A critical appraisal of Lewin's Prochlorophyta. Br. Phycol. J. 12: 271–276.

Technical Report Series

Technical Reports are research documents that are of sufficient importance to be preserved, but which for some reason are not appropriate for primary scientific publication. Inquiries concerning any particular Report should be directed to the issuing establishment.

Rapports techniques

Les rapports techniques sont des documents de recherche qui revêtent une assez grande importance pour être conservés mais qui, pour une raison ou pour une autre, ne conviennent pas à une publication scientifique de fonds. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

687. ROWELL, T. W., G. ROBERT, K. B. SWANSBURG, AND R. DAVIS. 1976. Soft-shell clam depuration Digby, Nova Scotia. Fish. Mar. Serv. Res. Dev. Tech. Rep. 687: 121 p.

Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 22¢ per page and a minimum charge of $2.20. Information on translations can be obtained from the Scientific Information and Publications Branch.

Traductions

Les traductions dont il est question ci-après ont été préparées par ou pour le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir des exemplaires, à 22¢ la page et à un prix minimum de $2.20 aux Services de traduction, Institut canadien de l'information scientifique et technologique, Conseil national de recherches du Canada, Ottawa (Ontario), K1A 0S2. La Direction de l'information et des publications scientifiques peut fournir des renseignements sur ces traductions.

SHEPELEV, A. P. 1977. The effect of acute hypothermia on the composition of mitochondrial lipids in the myocardium of warm-blooded animals. Fiziol. Zh. SSSR I.M. Sechenova. 63: 442–447. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 4151, 1977)

BURLAKOV, A. B., AND N. V. BELOVA (1); BURLAKOV, A. B. (2); BURLAKOV, A. B., AND N. I. BELYANOV (3). 1976. Comparative evaluation of the influence of some hormonal preparations on male silver carp (*Hypophthalmichthys molitrix*) (VAL.) (1). Some properties of gonadotropic hormones of carp — *Cyprinus carpio* L. (2). Specificity of gonadotropic hormones of the hypophysis of fish (3). Minist. Rybn. Khoz. SSSR Tsentr. Nauchno Issled. Inst. (illegible) Tekh. Ekon. Issled. (1): 143–146. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 4152, 1977)

SATO, S., M. KAYAMA, AND M. MASHIBA. 1974. Effects of environmental factors on the lipid components of *Porphyra* sp. Hiroshima Daigaku Sui Chikusangakubu Kiyo 13: 199–205. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 4153, 1977)

NOSKOV, A. S., AND V. A. RIKHTER. 1977. Instructions on the collection of size-age data. Tr. Atl. Nauchno Issled. Inst. Rybn. Khoz. Okeanogr. p. 9. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 4154, 1977)

TILGNER, D. J. 1977. Advances in smoking technology. Fleischwirtschaft 57: 45–50, 52. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4155, 1977)

BURLAKOV, A. B. (1); BURLAKOV, A. B., B. V. VERIGIN, AND A. P. MAKEEVA (2). 1976. (1) The taxonomic specificity of gonadotropic hormones of fish hypohyses. (2) The influence of gonadotropins and steroids of mammals on metabolism and the reproduction system of some cyprinids. Ekol. Fiziol. Ryb. Tezisy Dokl. III Vses. Konf. Part 2: 149–151. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 4156, 1977)

HAMM, R. 1977. Analysis of curing smoke and smoked foods. Fleischwirtschaft 57: 92–96, 99. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4157, 1977)

WELKE, R., AND R. GROPP. 1977. "Ludwig Turek" — first supertrawler for Rostock collective fishing combine. Seewirtschaft 9: 226–229. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4158, 1977)

JAKUPSSTOVU, S. H., AND I. RØTTINGEN. 1975. Investigations on capelin (*Mallotus villosus*) and sand lance (*Ammodytes* sp.) off western Greenland in June–July 1974. Fisk. Gang (11): 155–160. (Transl. from Norwegian by Fish. Mar. Serv. Transl. Ser. No. 4159, 1977)

TAKEUCHI, T., AND T. WATANABE. 1977. Requirement of carp for essential fatty acids. Studies on nutritive value of dietary lipids in fish — VIII. Nippon Suisan Gakkaishi 43: 541–551. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 4160, 1977)

CAPELLA, P., C. PAOLETTI, AND R. MATERASSI. 1977. A comparison between algal lipids and olive oil, with particular reference to their unsaponifiable fraction. Riv. Ital. Sostanze Grasse 54: 271–273. (Transl. from Italian by Fish. Mar. Serv. Transl. Ser. No. 4161, 1977)

KUEHNHOLD, W. W. 1969. Effects exerted by water-soluble constituents of crude oils and crude-oil fractions on the ontogenetic development of herring fry. Ber. Dtsch. Wiss. Komm. Meeresforsch. 20: 165–171. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4162, 1977)

MARANESI, M., V. BARZANTI, P. L. BIAGI, AND E. TURCHETTO. 1976. Experimental observations showing different rates of absorption of rapeseed oil by rats of different age. Boll. Soc. Ital. Biol. Sper. 52: 517–522. (Transl. from Italian by Fish. Mar. Serv. Transl. Ser. No. 4163, 1977)

List of Establishments

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ANNOUNCEMENTS / AVIS

Fourth International Congress of Parasitology (ICOPA IV)

This conference of The World Federation of Parasitologists, organized and sponsored by the Committee for Parasitology of the Polish Academy of Sciences and the Polish Parasitological Society, will be held from August 19 to 26, 1978 in the Palace of Culture and Science, Warszawa, Poland.

The scientific program will be divided into two main parts: Workshops and Plenary Review Sessions. Abstracts of short communications submitted by participants will be reproduced and distributed in advance. These will serve as the main basis for discussion in a Workshop, replacing the formal presentation of individual papers. The Plenary Review Sessions (relating to the nine Workshop Sections) will run consecutively so they can be attended by all participants of the Congress.

Inquiries and general suggestions should be directed to Secretary General of ICOPA IV, Prof. Dr B. Bezubik, Department of Parasitology, University of Warszawa, 00-927 Warszawa, Poland.

Suggestions and inquiries about the scientific program should be directed to Prof. Dr W. Ślusarski, Chairman of the Scientific Programme Committee of IOCPA IV, Research Centre for Parasitology, ul. L. Pasteura 3, S.p. 153, 00-973 Warszawa, Poland.

Quatrième Congrès international de parasitologie (ICOPA IV)

Cette conférence de la Fédération mondiale des parasitologistes, organisée et dirigée par le comité de parasitologie de l'Académie des sciences de Pologne et la Société de parasitologie de Pologne, se tiendra du 19 au 26 août 1978 au Palais de la culture et de la science de Varsovie, Pologne.

Le programme scientifique comprendra deux parties principales : des ateliers de travail et des séances plénières de revue. On distribuera à l'avance le résumé des brèves communications des participants. Ces résumés remplaceront la présentation formelle de travaux individuels et serviront de base à la discussion dans les ateliers de travail. L'horaire des séances plénières de revue (se rapportant aux neuf sections d'ateliers de travail) sera établi de façon que tous les participants au congrès puissent y assister.

On est prié d'adresser demandes de renseignements et suggestions d'ordre général au secrétaire général du ICOPA IV, M. le professeur B. Bezubik, Département de parasitologie, Université de Varsovie, 00-927 Varsovie, Pologne.

Les suggestions et demandes de renseignements relatives au programme scientifique devront être adressées à M. le professeur W. Ślusarski, président du comité du programme scientifique du ICOPA IV, Centre de recherche en parasitologie, ul. L. Pasteura 3, S.p. 153, 00-973 Varsovie, Pologne.

Guidelines for Surface Water Quality
(Development of the Guidelines and the Need for Input from Researchers)

The Water Quality Objectives and Standards Group of the Water Quality Branch of Environment Canada is under mandate to produce guidelines for the development of water quality objectives for Canada's inland waters.

The guidelines will present scientifically defensible recommended limits and a concept of their use in water resource management for the beneficial uses of water for raw public water supply, agriculture (livestock watering and irrigation), recreation and aesthetics, industrial water supply, and for the protection and preservation of aquatic life and wildlife. Criteria for all significant substances, particularly the persistent ones that might have a deleterious effect on the above uses, will be evaluated in terms of these water uses.

The purpose of the guidelines is to provide water managers with the scientific base for developing water quality objectives specific to their management areas. They are to be used in conjunction with local social and economic conditions, water use requirements, and local water quality conditions.

The scientific information to develop the recommended limits is being gathered from published and unpublished work and from direct consultation with researchers located primarily in North America. Topics include lethal and sublethal effects on aquatic flora and fauna, wildlife, crops, farm animals, and humans; metabolism of substances and metabolite toxicity; movement and persistence of substances through the aquatic and terrestrial environment; toxicity of mixtures of substances, as well as carcinogenesis, teratogenesis, mutagenesis; and bioaccumulation of substances.

Directives concernant la qualité des eaux de surface
(Établissement des directives et besoin de l'apport des chercheurs)

Le groupe des normes et objectifs de qualité des eaux de la Direction de la qualité des eaux d'Environnement Canada a été chargé de produire des directives qui permettront de définir des objectifs de qualité pour les eaux intérieures du Canada.

Les directives définiront des limites scientifiquement défendables et présenteront un concept de leur utilisation dans la gestion des ressources en eau. Elles viseront à assurer un usage profitable des eaux en vue de l'approvisionnement de la population en eaux non traitées, de l'agriculture (abreuvage des troupeaux et irrigation), de la récréation et de l'esthétique, de l'approvisionnement des industries, de même que pour la protection et la conservation de la vie aquatique et du gibier. On évaluera en fonction de ces usages de l'eau les critères applicables à toutes les substances importantes, en particulier les substances persistantes qui pourraient empêcher d'utiliser les eaux aux fins mentionnées plus haut.

Le but des directives est de fournir aux gestionnaires des eaux une base scientifique qui leur permettra de formuler des objectifs de qualité des eaux propres à leurs domaines de gestion. Elles devront être utilisées en fonction des conditions sociales et économiques locales, des besoins en eau et des conditions locales de qualité des eaux.

On est à recueillir les renseignements scientifiques sur lesquels fonder les limites à recommander. Ces renseignements proviennent de travaux publiés et inédits, et de consultations directes avec les chercheurs d'Amérique du Nord d'abord. Parmi les sujets couverts, on compte : les effets létaux et sublétaux sur la flore et la faune aquatique,

Documents have been completed for mercury, chromium, cadmium, selenium, nickel, arsenic, and silver. The metals copper, lead, and zinc; pesticides; industrial organics; and nutrients will be reviewed in the near future. The references used in developing the recommended limits are stored on a computer file. Reference lists can be obtained by interested persons by contacting the Water Quality Branch.

Reports of relevant laboratory experiments and their extension to the field for verification under natural environmental situations have been scarce. Such results would be of direct applicability in the recommendation of permissible levels of substances to various water uses including the safeguarding of the aquatic environment. Presently, this usually large information gap necessitates the use of application factors to arrive at recommended safe levels and the safety margin allowed by these is not always well understood.

Our group would be pleased to receive information regarding current research on the effects of polluting substances. Any ideas on the use of application factors and how currently available toxicity information can be applied to the field situation for the determination of safe levels would be welcome.

M. C. Taylor, A. Demayo, K. W. Taylor, E. D. Brien, S. W. Reeder
Water Quality Branch, Inland Waters Directorate, Environmental Management Service, Environment Canada, Ottawa, Ont. K1A 0E7

le gibier, les récoltes, le bétail et l'homme; le métabolisme des substances et la toxicité des métabolites; le mouvement et la persistance des substances dans les milieux aquatiques et terrestres; la toxicité des mélanges de substances, de même que la carcinogénèse, la tératogénèse, la mutagénèse; et la bioaccumulation des substances.

Nous avons complété les documents relatifs au mercure, chrome, cadmium, sélénium, nickel, arsénic et argent. Nous ferons sous peu la revue des métaux cuivre, plomb et zinc; des pesticides; des composés organiques industriels; et des éléments nutritifs. Les références utilisées dans l'établissement des limites recommandées sont stockées sur fichier mécanographique. Les personnes intéressées pourront se procurer des listes de références en s'adressant à la Direction de la qualité des eaux.

Les rapports d'expériences de laboratoire pertinentes et de leur vérification sur le terrain dans des conditions ambiantes naturelles ont été rares. De tels résultats seraient directement applicables lorsqu'il s'agit de recommander des niveaux permissibles de substances pour divers usages des eaux, y compris les niveaux qui assureront la sauvegarde de l'environnement aquatique. Dans le moment, à cause de cette lacune généralement importante dans nos connaissances, il nous faut utiliser des facteurs d'application pour en arriver à des niveaux sûrs recommandés. La marge de sûreté permise par ces facteurs n'est pas toujours bien comprise.

Notre groupe recevra avec plaisir tous renseignements concernant la recherche en cours sur les effets des polluants. Toute idée sur l'emploi de facteurs d'application et sur la façon dont nos connaissances actuelles sur la toxicité peuvent être appliquées à des situations sur le terrain dans la détermination de niveaux sûrs sera la bienvenue.

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Plains Aquatic Research Conference

This conference, meant to appeal not only to researchers but also to professionals and paraprofessionals, will take place for 2 days in late summer/early autumn 1979 at the University of Regina. It will be devoted to aquatic research and its application in the plains environment. The conference is sponsored by the Water Studies Institute, Canadian Plains Research Centre and the Department of Extension, University of Regina.

The purpose of the conference is to provide a communication vehicle among those doing research in this special environment; to provide for communication between researchers and practitioners; and to provide a "state-of-the-art" statement on one particular problem of broad importance in the plains environment. There will be five consecutive sessions of contributed papers and a symposium entitled "The ideal plains surface water supply; its biology, chemistry, physics, design and economics."

Preliminary responses expressing an interest in the conference are requested. Please write to Department of Extension, University of Regina, Room 113, College Building, College Avenue and Scarth Street, Regina, Sask. S4S 0A2.

Conférence sur la recherche aquatique sur les plaines

Cette conférence, qui devrait intéresser non seulement les chercheurs mais aussi les professionnels et les paraprofessionnels, occupera 2 jours à la fin de l'été ou au début de l'automne 1979 à l'Université de Régina. Elle portera sur la recherche aquatique et son application à l'environnement des plaines. Cette conférence est organisée par l'Institut d'études sur les eaux, le Centre de recherche sur les plaines canadiennes et le Département d'expansion de l'Université de Régina.

Le but de la conférence est de fournir un forum aux chercheurs travaillant sur cet environnement spécial; de permettre aux chercheurs et aux praticiens de communiquer entre eux; et de faire le point sur l'état de nos connaissances sur un problème particulier touchant l'environnement des plaines. Il y aura cinq séances consécutives au cours desquelles seront présentées des communications et un symposium intitulé : « L'approvisionnement idéal en eaux de surface des plaines; biologie, chimie, physique, design et économique ».

Nous voudrions avoir un aperçu préliminaire de l'intérêt que suscite cette conférence. On est prié d'écrire au Department of Extension, University of Regina, Room 113, College Building, College Avenue and Scarth Street, Regina, Sask. S4S 0A2.

Bibliography of Canadian Aquatic Invertebrates
(*Request for Information*)

As an expansion of "A Synopsis of Canadian Marine Zooplankton" (Bull. Fish. Res. Board Can. 176, 1971), the Canadian Aquatic Identification Centre is compiling a bibliography of Canadian aquatic invertebrates. We intend to list all species reported from Canadian and contiguous waters, and all names applied to these species. References to the species will be abstracted as to general locality, subject matter, synonyms, taxonomic grouping, and habitat.

After nearly 3 years of work, we have collected 14 000 names applied to Canadian invertebrates, excluding Protozoa, Insecta, and endoparasites, but including the lower Chordata. The entry of all information into a computer is being planned. It is hoped that this information will become available to the public when computerization is complete.

Searching of all references pertaining to Canadian species, particularly in such fields as physiology, biochemistry, cell biology, and pollution biology, has become very difficult, and we are therefore asking for help from authors. We would like information on all publications pertaining to Canadian species, preferably in the form of reprints, but any relevant reference list will be much appreciated. We would also be very grateful for access to unpublished bibliographies on any aspect of Canadian aquatic invertebrates.

In return, we can offer information available through our system. This service will be very limited before completion of computerization, because we do not have the staff to do any extensive search. Our system, however, is available to anyone wishing to make use of it in person. Please contact D. R. Laubitz or C. T. Shih, Canadian Aquatic Identification Centre, National Museum of Natural Sciences, Ottawa, K1A 0M8.

Bibliographie des invertébrés aquatiques canadiens
(*Demande de renseignements*)

Le Centre canadien Aquatique est à compiler une bibliographie des invertébrés aquatiques canadiens comme complément à la publication « A Synopsis of Canadian Marine Zooplankton » (Bull. Fish. Res. Board Can. 176, 1971). On se propose d'y inclure toutes les espèces signalées dans les eaux canadiennes et contiguës, ainsi que tous les noms donnés à l'espèce. Chaque espèce sera l'objet d'un résumé analytique comportant la localité, le sujet traité, les synonymes, le classement taxonomique et l'habitat.

Commencé il y aura bientôt 3 ans, le travail comprend déjà 14 000 noms d'invertébrés canadiens, excluant les Protozoaires, les Insectes et les endoparasites, mais incluant les Chordés inférieurs. On se propose d'introduire tous les renseignements dans un ordinateur. Le public, espère-t-on, aura accès à ces données une fois l'informatisation terminée.

Les recherches bibliographiques se rapportant aux espèces canadiennes, surtout dans des domaines telles la physiologie, la biochimie, la biologie cellulaire et la biologie de la pollution sont devenues très difficiles. C'est pourquoi nous demandons l'aide des auteurs. Nous désirerions recevoir des renseignements sur toutes les publications canadiennes, de préférence sous forme de tirés à part. Toute liste de références pertinentes sera toutefois très appréciée. Nous serions aussi reconnaissants d'avoir accès aux bibliographies inédites se rapportant à un aspect quelconque des invertébrés aquatiques canadiens.

En retour, nous sommes en mesure de fournir les renseignements disponibles grâce à notre système. Ce service sera très limité tant que l'informatisation ne sera pas terminée : notre personnel limité ne nous permet pas des recherches poussées. Notre système est cependant à la disposition de quiconque désire l'utiliser en personne. On est prié de communiquer avec D. R. Laubitz ou C. T. Shih, Centre canadien Aquatique, Musée national des sciences naturelles, Ottawa, K1A 0M8.

Water Pollution Publications Available from the National Research Council of Canada

The Water Subcommittee of the National Research Council of Canada (NRCC) Associate Committee on Scientific Criteria for Environmental Quality has published the following reports regarding problems in water pollution. These are available from Publications, NRCC, Ottawa, Canada K1A 0R6. For information regarding forthcoming publications on water pollution contact Dr R. C. Pierce, Environmental Secretariat, Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6.

1. The Effects of Pulp and Paper Wastes on Aquatic Life with Particular Attention to Fish and Bioassay Procedures for Assessment of Harmful Effects by J. R. Marier, Division of Biological Sciences, National Research Council of Canada, Ottawa, Ont. K1A 0R6. (*$2.50; Publication No. NRCC 13501*)

The pulp and paper topic, given first priority by the Water Subcommittee, is of foremost importance to Canada and the Subcommittee's concern was influenced by the fact that water usage in pulp and paper production is enormous: in 1966, 560 billion gallons were used and this is expected to rise to 720 billion gallons by 1980 and to 1020 billion gallons by the year 2000. Although considerable information has been published on pulp and paper wastes, it is highly dispersed in the technical literature and needed to be assessed and presented in an integrated manner.

This report contains five major segments: 1. characteristics of various types of water and their influence on the impact of various pollutants; 2. production of wastes by the various mill processes; 3. substances present in pulpmill wastes; 4. adverse environmental effects of pulpmill wastes; 5. various tests that can be used to assess undesirable effects on aquatic ecosystems.

This report is aimed particularly at highlighting data that showed a gradation of effects caused by varying amounts of any pollutant and by different lengths of exposure to these substances. Special emphasis is placed on conditions existing within nature, although references to laboratory studies are also included. It is important to emphasize cause-vs.-effect type of information if we are to develop a sound scientific basis for the protection of the environment, which is ultimately man's vital life-support system.

One of the problems discussed in the report is dissolved oxygen. Just as human beings are dependent on the oxygen present in the air they breathe, so fish and other aquatic life are dependent on the small amount of dissolved oxygen present in the water. Pulp and paper wastes require oxygen for decomposition; the higher the amount of such wastes, the greater will be the demand for oxygen from the receiving waters and the greater the stress imposed on all aquatic organisms. Another type of burden can be imposed by discharging wastes that are either very alkaline or very acidic and this pH factor is also discussed in the report. Yet another type of problem is created when large volumes of wastes containing appreciable quantities of suspended wood fiber are discharged; the fibers can eventually "blanket" the bottom of a waterway, resulting in suffocation of bottom-dwelling life. Pulp and paper wastes can also contain several substances that are directly poisonous to sensitive aquatic organisms; the report presents data bearing on this toxic aspect of the problem.

These factors and others can act simultaneously to adversely affect an aquatic ecosystem. Hence, the consideration of each factor separately can often be inadequate. For this reason, many researchers are convinced that the total impact of pulp and paper wastes can best be evaluated by recourse to a laboratory test in which a responsive aquatic organism is exposed to the actual wastes. The report discusses features of such bioassay tests and points out that the conditions in these tests are often not as severe as those in the actual outdoor environment. The report emphasizes that the "ecological health status" of the receiving waters is the important factor, rather than mere reliance on the composition of wastes in the outfall pipes.

Recommendations concerning various water quality factors are given at the end of the report. However, if there is one overall need, it is the establishment of a coordinated water quality "monitoring grid" covering all of Canada's major waterways. This would permit on-going assessment of their ecological health status, not only as affected by pulp and paper wastes, but also by all other forms of discharge.

2. The Application of the Phosphorus Loading Concept to Eutrophication Research by R. A. Vollenweider, Department of Fisheries and the Environment, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6 and P. J. Dillon, Ontario Ministry of the Environment, Water Resources Branch, Limnology and Toxicity Section, Rexdale, Ont. M9W 5L1. (*$1.25; Publication No. NRCC 13690*)

Almost everyone is familiar with the problem posed by the process of overfertilization or eutrophication of lakes (for example, Lake Erie). In such situations, the lakes receive an excess supply of nutrients, and this can lead to an overproduction of weeds and algal organisms which can suddenly appear as "blooms." Aside from being a nuisance, these algal organisms and weeds can deplete the life-giving oxygen content of the lake water when they decay, setting off a chain of events which can seriously affect the quality of the water. In fact, a lake that was once "crystal clear" or oligotrophic can deteriorate to a mesotrophic, eutrophic, or even hypereutrophic state.

For the past few years, researchers have focused attention on the factors responsible for this deteriorating process, and one of the concepts most widely used was based on the work of Dr R. Vollenweider. After detailed intercomparison of the many factors in such trophic alterations of lakes throughout the world, Dr Vollenweider demonstrated that a "loading rate" concept could be utilized to assess the situation, and could also be used to predict the reversibility of such processes when input was reduced. In this concept, the critical factors are the phosphorus input to the lake as related to the lake's area, depth, and the time needed for the lake water to be completely exchanged. Phosphate was identified as the most critical of the nutrients, and also as the nutrient that is potentially the easiest to control. Dr Vollenweider has demonstrated that a reduction of phosphorus input can reverse the undesirable effects and can lead to a progressive improvement in the trophic status of the lake. This approach also offers a basis by which valuable cause/effect data can be obtained regarding the trophic status of aquatic ecosystems in general.

This topic was identified as a priority one by the Water Subcommittee, which requested Dr Vollenweider to super-

vise the preparation of a concise report outlining his "loading rate" concept. This, Dr Vollenweider has done with the cooperation of Dr P. J. Dillon. Of necessity, the report is of a highly technical nature, primarily directed to the specialists in the field. This timely report provides scientists with a means of deriving the necessary cause/effect data for assessing the trophic state of aquatic ecosystems so that present and future trends can be characterized.

3. Environmental Cause/Effect Phenomena Relating to Technological Development in the Canadian Arctic by W. Eedy, Beak Consultants Ltd., 6870 Goreway Dr., Mississauga, Ont. L4V 1L9. (*$2.00; Publication No. NRCC 13688*)

This report emphasizes environmental cause/effect interrelations observed as a consequence of man-mediated disruptions in arctic regions. The report includes coverage of the available scientific literature that came to the author's attention prior to February 8, 1974.

An attempt has been made to make the document as comprehensive a cause/effect compilation as possible. Wherever possible, quantifiable data are presented; also, an attempt is made to identify areas of research where knowledge is lacking or seems to be insufficient. What might be called "basic ecological interrelations" are discussed only in relation to the document's emphasis on environmental alterations induced by man's technological intervention. Accordingly, this report should not be read as an all-encompassing treatise on the fundamental ecology of arctic regions.

The document summarizes temperature effects on toxicity, effects of chemicals, air pollution, sewage and wastes, and terrain damage. This is followed by the detailed technical report, with the various phenomena described on a region-by-region basis dealing with, in turn, the western Arctic Islands, the eastern Arctic Islands, the Yukon Territory, Alaska, the Mackenzie River Region, the eastern Northwest Territories, and northern Quebec. There is, in addition, a bibliographic section listing general and theoretical reviews. For the reader's convenience a subject index is also included.

4. Waterborne Dissolved Oxygen Requirements and Criteria with Particular Emphasis on the Canadian Environment by J. C. Davis, Department of Fisheries and the Environment, Fisheries and Marine Service, Pacific Environment Institute, West Vancouver, B.C. V7V 1N6. (*$2.00; Publication No. NRCC 14100*)

Reductions in the level of oxygen available to fish have a marked effect on many of their physiological, biochemical, and behavioral processes. This report identifies the oxygen levels at which such adverse effects first become apparent. These cause/effect relations based on current knowledge are used to estimate the harm (or risk) commitment.

An adequate oxygen supply for metabolic processes including swimming, migrating, and feeding is necessary for the survival of healthy fish populations. Effects of hypoxia, an oxygen deficiency to the body tissues, can include reduction of maximum swimming speed, avoidance behavior, and altered respiration and heart rate. Deformities may occur and there may be a decrease in growth rate. The latter is an expression of the net product of metabolic function and as such, constitutes a useful criterion for permissibly low oxygen levels.

Apparently low oxygen in the presence of toxic agents enhances their lethal effect on some fish. This has been noted for zinc, lead, copper, and phenols.

The document lists the incipient oxygen response thresholds for various fish groups and habitats gathered from the literature, with emphasis on Canadian species, and describes the rationale for choosing the reported response and its significance. Response levels reported are incipient oxygen levels where effects first become apparent, thus providing a biological indication of the onset of hypoxic stress.

5. The Effects of Petroleum Refinery Liquid Wastes on Aquatic Life, with Special Emphasis on the Canadian Environment by R. P. Côté, Department of Fisheries and the Environment, Environmental Protection Service, Water Surveillance Unit, Halifax, N.S. B3J 3E4. (*$2.00; Publication No. NRCC 15021*)

This report attempts to assess the impact of waterborne petroleum refinery wastes on aquatic ecosystems. The document deals exclusively with this subject and does not include the topic of "Oil Spills" from tankers, etc.

In Canada, there are specific petroleum refineries whose effluents are highly toxic to fish; however, the majority of refinery effluents tend to be moderately or not acutely lethal. In many cases, petroleum refineries do not contribute large volumes of waste waters to the environment, and there is a continuing trend towards a reduction in effluent volume.

Some of the major effluent components are known to be highly toxic under certain circumstances. One of the difficulties in assessment is that the toxic components are discharged in complex mixtures that can contain phenols, sulfides, heavy metals, etc., and that vary according to specific refinery operations.

Refinery operations may create and discharge carcinogenic compounds, cumulative toxicants, and compounds that can affect the sensory perception of aquatic organisms, in addition to affecting the taste and odor of fish flesh.

Since refinery effluents are generally discharged into areas already receiving one or more wastes, it is not always possible to determine and assess the specific effects of refinery effluents on the environment under actual field conditions. It then becomes important to conduct toxico-pharmacological studies, to diagnose those effects in the environment that are relatable to specific causes.

6. NTA (Nitrilotriacetic Acid) — An Ecological Appraisal by A. Prakash, Department of Fisheries and the Environment, Environmental Protection Service, Water Pollution Control Directorate, Ottawa, Ont. K1A 0H3. (*$1.25; Publication No. NRCC 15023*)

Ever since nitrilotriacetic acid has been used in detergents in North America, it has constantly been under a cloud of suspicion and uncertainty with regard to its environmental acceptability. This report emphasizes the environmental aspects of NTA and includes an assessment of the ecological distribution and effects of NTA in the aquatic ecosystem, based on data from field monitoring, laboratory and pilot-scale studies in Canada and the United States. Factors affecting biodegradability of NTA in the recipient environment, and current and projected environmental levels of NTA, are discussed. General effects of increased amounts of NTA on aquatic ecosystems and specific effects on algae, invertebrates, and fish are reviewed. The report concludes that adequate safety margins exist between anticipated NTA levels in the environment and those that are likely to produce deleterious ecological effects, but it recommends continued vigilance in certain specific situations where a potential for cumulative buildup of NTA may exist.

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Abbreviations Used Frequently in the *Journal*

Abbreviate terms denoting units of weight and measurement in the text only when preceded by numerals.

centimetre	cm
centimetre, square	cm^2
centimetre, cubic	cm^3
centimetre per gram per second	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
day	d
decimetre	dm
degree Celsius	°C
degrees of freedom	df
electronvolt	eV
farad	F
gram	g
hectare	ha
hertz	Hz
hour	h
joule	J
kilometre	km
litre	L
lumen	lm
lux	lx
metre	m
metre, square	m^2
metre, cubic	m^3
micrometre	μm
milligram	mg
millilitre	mL
millimetre	mm
millimetre, square	mm^2
minute	min
molar	M
mole	mol
month	mo
second	s
standard deviation	SD
standard error	SE
tonne	t
volt	V
volume	vol
watt	W
week	wk
year	yr

Abréviations fréquemment employées dans le *Journal*

Les termes représentant des unités de poids et de mesure dans le texte que lorsqu'ils sont précédés d'un nombre.

an	an
année	a
centimètre	cm
centimètre carré	cm^2
centimètre cube	cm^3
centimètre par gramme par seconde	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
décimètre	dm
degré Celsius	°C
degrés de liberté	dl
écart type	EcT
électrovolt	eV
erreur type	ErT
farad	F
gram	g
hectare	ha
hertz	Hz
heure	h
joule	J
jour	jour
kilomètre	km
litre	L
lumen	lm
lux	lx
mètre	m
mètre carré	m^2
mètre cube	m^3
micromètre	μm
milligramme	mg
millilitre	mL
millimètre	mm
millimètre carré	mm^2
minute	min
mois	mo
molaire	M
mole	mol
seconde	s
semaine	sem
tonne	t
volt	V
volume	vol
watt	W

Journal of the Fisheries Research Board of Canada

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